HYPOTHALAMIC-HYPOPHYSIAL INTERRELATIONSHIPS

A Symposium

HYPOTHALAMIC-HYPOPHYSIAL INTERRELATIONSHIPS

A Symposium
Third Annual Scientific Meeting of the
Houston Neurological Society
Texas Medical Center, Houston, Texas

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FOREWORD

THE Houston Neurological Society was founded in 1951 by a group interested in fostering a closer relationship between those working in clinical and basic science disciplines allied to Neurology. It was our hope that the Society would create the stimulus needed for interest in both teaching and research in this field Each year, since 1952, the Society has sponsored an Annual Scientific Meeting including a symposium on some subject of broad current interest

For the 1955 meeting the topic "Hypothalamic-Hypophysial Interrelationships" was selected because of the increasing interest in the relationship of neurology and neurophysiology to endocrinology. The literature in this field has been rapidly expanding and it was considered that a critical appraisal of the current status of our knowledge would be timely.

In large measure the successful grouping of the presentations and the freedom of the ensuing discussions were directly attributable to the guidance of Dr. Hebbel E Hoff in his capacity as chairman

The symposium could not have taken place without the many contributions, both financial and otherwise, made by the following.

Baylor University College of Medicine.
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We wish also to express our appreciation for the great amount of time and effort expended by Drs. Charles A. Carton, William S. Fields, and Roger Guillemin in assisting in the planning of the program and editing of these proceedings.

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CONTENTS

HYPOTHALAMIC-HYPOPHYSIAL INTERRELATIONSHIPS

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NEURAL PATHWAYS TO THE HYPOPHYSIS*

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THE MECHANISM of nervous control of the pituitary gland presented a difficult problem for many years On the one hand the neurohypophysis was known to contain abundant nerve fibers, but seemed to lack secretory cells, and on the other, although the pars distalis of the adenohypophysis was known to contain a variety of secretory cells, most histologists agreed that nerve fibers were scanty or absent Ranson and his collaborators (Fisher, Ingram and Ranson, 1936) in this country and Verney (1926) in England clearly demonstrated that the neurohypophysis was capable of secretion and was controlled by the supraoptic and paraventricular nuclei. Harris (1947) showed that direct stimulation of the neural lobe or neural stalk caused the liberation of posterior pituitary hormones and to some (Fulton, 1949) the problem of the neurohypophysis appeared solved by the work of Gersh (1939) on pituicytes. This latter did not go unchallenged on anatomical (Rasmussen, 1938, Green, 1947a) and experimental grounds (deRobertis and Primavesi, 1942), and the alternative hypothesis that the abundant nerve fibers were responsible for the secretions of the neural lobe was advanced (Rasmussen, 1938). This gained impetus with the discoveries of Bargmann and his collaborators who observed sharp staining of the neurohypophysis by chrome alum hematoxylin. They proposed that the curious colloid described in the hypothalamic nuclei and elsewhere by the Scharrers was identical with the substance so stained

* This work was supported in part by Grant B-610 from the United States Public Health Service

and was in fact the hormone or a hormone 'carrier.' The work of many authors now leaves little doubt that a relationship exists between the so-called Gomori substance and the antidiuretic hormone, though some points of an equivocal nature still remain (Green and Van Breemen, 1955).

The control of the pars distalis represents another problem. Various authors proposed a humoral transmitter from the tuberal region or neurohypophysis as a possible controlling mechanism for the pars distalis (Hinsey and Markee, 1933, Harris, 1944). The hypothesis was put forward on the basis of anatomical studies and various data collected from the literature that the hypophysial portal circulation served to carry a transmitter, liberated by nervous action in the median eminence, to the pars distalis there to influence the activity of this part of the gland (Green and Harris, 1947). Subsequently, experimental evidence (Harris, 1949, Harris and Jacobsohn, 1952) showed that this was apparently true for gonadotrophins Harris (1955) indicates that some but not all phases of ACTH and TSH secretion are also controlled through the portal vessels.

Anatomical studies (Green, 1951a) indicated that the hypophysial portal circulation or a functionally similar system of vessels is constant in some 80 representative species of vertebrates from cyclostomes to man Furthermore, in some species, for example, the pigeon, known to be particularly sensitive to nervous influences on the pituitary the only apparent link between the hypothalamus and the pars distalis is via a leash of blood vessels penetrating a dural septum between adeno- and neurohypophysis (Green, 1951a) Using phase contrast illumination and media of different refractive indices, strong evidence was obtained to show that the pericellular nets of so-called nerve fibers in the pars distalis were, in fact, reticular connective tissue (Green, 1951b).

The portal vessels have recently received particular attention in man (Green, 1948; Xuereb, Prichard and Daniel, 1954a, b) and the latter authors have published beautiful photographs of latex injections giving the clearest of all demonstrations of their anastomoses. This system is constant from the caudata to man and below the caudata is slightly modified in aquatic ver-

tebrates which lack a neural lobe, but are often provided with a large median eminence and neural stalk The neural lobe appears to have developed *pari passu* with the acquisition of a land habitat (Green, 1951a). Possibly this may be correlated with a new need to stabilize the tonicity of the body fluids associated with life on dry land.

The hypothalamus as such has no direct vascular connection with the pituitary, as was first shown by Wislocki and King (1936). Though certain regions, notably the supraoptic and paraventricular nuclei, receive an extraordinarily rich blood supply, possibly associated with osmo-reception, this has no anastomoses with the hypophysial system of vessels The blood supply of the neural lobe is quite independent of that of any other part of the pituitary gland also.

Many environmental factors as well as psychic phenomena affect the activity of the hypophysis cerebri and both the neurohypophysis and adenohypophysis are affected by influences mediated through a wide variety of receptors. These exteroceptive factors have been reviewed repeatedly (Marshall, 1936, 1942, Green, 1946, 1947b, Harris, 1948). Although little is known about olfactory or proprioceptive influences affecting the anterior pituitary, it seems very likely that they may play some role in controlling its production of hormones, and it is clear that in one species or another all other modalities of afferent stimulation can affect this part of the gland

Marshall (1936, 1942) suggested that courtship and sex display played a role in pituitary stimulation, and since this time many examples of psychic influences on the hypophysis have been reported. Perhaps the most dramatic of these is the narcissistic behavior of the female pigeon with a mirror (Matthews, 1939), but ancient clinical observations on emotion and the menstrual cycle must be placed in the same category as well as the many recent studies on emotional stress.

While the list for the neurohypophysis is less impressive, it is growing, and it is clear that emotional responses occur and that exercise, suckling, for, changes in blood tonicity and stress all affect the output of its hormones.

Since the evidence for direct sympathetic or parasympathetic

control of the pars distalis is inconclusive (Harris, 1948) and evidence for neurohypophysial control by this route even more equivocal, it is clear that the main burden of pituitary control must be carried via the hypothalamus and the hypophysial stalk linking it to the pituitary.

Thus, there is abundant evidence for a truly amazing convergence of nervous influences in this area. We know that the neural stalk contains a variety of fibers derived from the hypothalamus In the rhesus monkey and the cat the following main categories can be recognized (Green, 1951a): (1) fibers derived from the supraoptic and paraventricular nuclei; (2) fibers from the general area of the medial forebrain bundle, and (3) fibers from the lateral tuberal nuclei. In addition, small contributions from the periventricular system and the nucleus suprachiasmaticus may be observed, but no direct connections with the mammillary body.

While the demonstration by Ranson and his colleagues that the supraoptic and paraventricular nuclei control neurohypophysial secretion and the studies of Bargmann, Scharrer, Ortmann, Hild and others (Scharrer and Scharrer, 1954) indicate that these nuclei may also produce the hormones, the precise groups of cells involved in anterior pituitary control are not well understood, despite innumerable descriptions of dysfunction following clinical and experimental lesions and many examples of altered function following hypothalamic stimulation. It may well be that in the case of the pars distalis we are dealing with hypothalamic fibers rather than cells Thus the position is that we know the peripheral receptors, but are very ignorant of pathways within the nervous system between these receptors and the final common neural path in the tractus hypophysius prior to the hypophysio-portal circulation

Admitting from the start that these connections are not known, it may be appropriate to speculate about some of the neural pathways which may play a role in linking the anterior pituitary with peripheral receptors and the outside world. To try to clarify this discussion, Figure 1 illustrates certain possible connections Within the system of cells and fibers concerned in the regulation of anterior pituitary activity, the following characteristics might

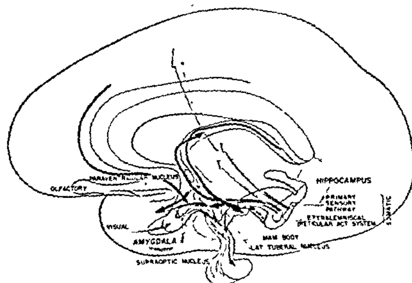


Figure 1. Illustration of pathways discussed in text to account for convergence of afferents onto the tuberal region The primary sensory pathways are indicated light gray as is the hypophysio-portal system Proposed pathways under discussion dark gray Possible convergent pathways medium gray Amygdala, hippocampus and hypothalamic nuclei, stippled gray

be expected. (1) They should be phylogenetically old. (2) Many modalities of impulses must converge upon them (3) They must be related to "higher" functions also since, for example, adaptation to psychic stress occurs and emotional influences play an important role as indicated above (4) They must have properties of discrimination (for example, the stimuli of a rectal thermometer and coitus evoke different anterior pituitary responses). (5) Finally, prolonged hypothalamic stimulation is required to evoke anterior pituitary effects suggesting that prolonged after-discharges must be produced by afferents reaching the hypothetical system of neurons.

Perhaps the most obvious point of departure would be to consider the primary lemniscal pathway While this might conceivably relay in the thalamus and cerebral cortex and then in-

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monkey and that in the rabbit and cat this is probably through the route: extralemniscal afferents→lateral hypothalamus→septum →pre-commissural fornix→hippocampus→post-commissural fornix→mammillary body→mammillo-thalamic tract→anterothalamus→cingulum. This also would be an attractive alternative, but against it must be set the absence of evidence for direct connections between the mammillary body and the pituitary and the suggestion implied by this lack of evidence is that the impulses sweep through the hypothalamus into the anterior thalamus. On the other hand, it has been shown (Green and Arduini, 1954) that the hippocampal responses show adaptation analogous to that of the neo-cortex and the hippocampus is, of course, an ancient and distinguished part of the cerebral hemisphere which might qualify as the dean of cortical areas. Indeed, Herrick (1933) suggested that its role was to correlate visceral and somatic activities.

Another region of the forebrain which plays an undoubted role in visceral activity is the amygdala. Bilateral removal of the region of the temporal lobes, especially the regions of the hippocampus and amygdala, produced hypersexuality and abnormal sexual behavior (Kluver and Bucy, 1939, Gastaut, 1952; Schreiner and Kling, 1953, 1954, see also Klüver, 1952). Although this never has been directly correlated with hypothalamic and pituitary activity, Kluver and Bartelmez (1951) found changes in the ovaries and endometrium strongly suggestive of such a relationship, and Schreiner and Kling (1954) find that castration prevents the hypersexuality seen after lesions of the amygdala, but that it may be restored with testosterone. The wealth of connections between the amygdala and diencephalon makes such a role a very attractive one to consider Sawyer (1955) has recently found that changes in blood sugar produced by intravenous injection or by administration of insulin produce marked changes in the electrical activity of this structure. The main obstacle to this concept might appear to be the lack of evidence of afferents to the amygdala. Koikegami *et al.* (1954) produced ovulation in the rabbit by stimulation of the amygdala

Using single shock stimulation and the after-discharge technique, Arana *et al* (1955) were unable to locate afferent path-

fluence the hypothalamus from some cortical area, perhaps the frontal cortex, there are several reasons for thinking that this is not likely despite the observations of Beach (1948). Phylogenetically the neocortex is a recent acquisition and together with its associated thalamic nuclei is a mere parvenu compared with the pituitary and ventral diencephalon. Furthermore, this pathway could not account for the effects of visual stimuli, for even the analogous path is not involved. LeGros Clark, *et al* (1939) showed that in the ferret neither the visual cortex nor the bulk of the lateral geniculate were necessary for the estrus reaction of the ferret to constant illumination. They proposed that either the ventral nucleus of the lateral geniculate or the accessory optic tracts (by way of the subthalamus) relayed the stimulus to the hypothalamus. Since section of the optic nerve abolishes the response while almost complete destruction of the geniculate does not, their results certainly suggest that the impulse must leave the optic tract at some point before it arrives at the lateral geniculate.

The recent trend in neurophysiology has been to refer many adaptive and correlative mechanisms to subcortical areas in the brain stem, notably to the reticular activating system and to the non-specific thalamic nuclei Through these structures course afferent neurons which are derived from many sources and, at the same time, appear more complex in their connections than those of the direct lemniscal pathway. Phylogenetically this is an ancient collection of neurons, for it was present long before the neo-cortex developed. It is attractive to consider since it is prolonged rostrally into the posterior hypothalamic nuclei On the other hand, it appears that lesions in the anterior hypothalamus and preoptic region may affect certain anterior pituitary responses, a finding which would suggest either that these impulses would have to pursue a recurrent course or that in these particular cases they were not involved Very long after-discharges have not been recorded from these neurons in spite of their complex connections and behavior

Recently we have obtained evidence (Green and Arduini, 1954; Green and Machne, 1954, Green and Adey, 1955) that afferent impulses reach the hippocampus in the rabbit, cat, and

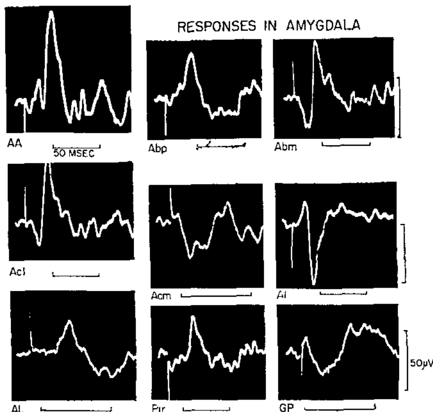


Figure 2 [illegible] stimulation [illegible] tions as inc [illegible] AA, anterior amygdala, Abp, N amygdaloideus basalis, pars parvocellularis, Abm, N amygdaloideus basalis, pars magnocellularis, Acl, N. amygdaloideus centralis, pars lateralis, Acm, N amygdaloideus centralis, pars medialis, AL, ansa lenticularis, Pir, lobus piriformis, GP, globus pallidus

1953, Green and Arduini, 1954). The hippocampus shows adaptation to emotional stimuli It has long been suspected to play a role in correlating visceral and somatic activities While the amygdala has abundant demonstrable connections with the hypothalamus, the hippocampus, which projects to it, is apparently receptive to all modalities of afferent stimulation The hippocampus must perform some correlative function in receiving and distributing the nervous impulses which pass to it via the extralemniscal pathways Adaptation is known to occur here and while

ways from the midbrain to the amygdala although, on the other hand, Machne and Segundo (1955) using microelectrodes have found evidence of profound and long-lasting changes in neuronal activity in the amygdala following peripheral stimulation. Their preliminary results also suggest that primary responses are difficult or impossible to record by the macroelectrode method Thus it might be suggested that the amygdala does not receive impulses directly, but only after considerable elaboration so that the effects of the initial volley are broadened into diffuse neuronal responses.

A possible mechanism whereby the amygdala might receive afferents is suggested by Figure 2. Single shock stimuli of the ipsilateral hippocampus evoke short-latency responses in the amygdala, particularly in the areas of the anterior, central and basolateral nuclei (Green and Adey, 1955), and this has been confirmed by strychnine neuronography (Clemente, Green and Sutin, 1955). Similar responses on the contralateral side have not been seen. Figure 2 illustrates some of these responses and responses in adjacent areas

The suggestion is made, therefore, that the rather complex pathway: peripheral afferents→extralemniscal afferent paths in the reticular activating system*→lateral hypothalamus→septum →hippocampal formation→amygdala→hypothalamus (by way of the stria and medial forebrain bundle) fulfills all the requirements for a system of neurons controlling the anterior pituitary, and possibly the posterior pituitary also. A second possibility is that direct relays from the septum to the amygdala may occur Responses in this area to afferent stimulation have been described (Green and Arduini, 1954, Hernández-Peón, Gunn and Eliasson, 1955)

All parts of the amygdala-hippocampal system are phylogenetically ancient Within it, many different afferents are correlated and an important role in emotional and visceral reactions is undoubtedly played by it It is capable of sustained after-discharges (Kaada, 1951, Green and Morin, 1953, Green and Shimamoto,

* It may be noted that the area from tegmental reticular activating system to septum may be regarded as a rostral extension of the reticular activating system of Moruzzi and Magoun (1949), since stimulation of this region produces the same neocortical "arousal" response (Green and Arduini, 1954) and lateral hypothalamic lesions produce sleep (Ranson, 1939)

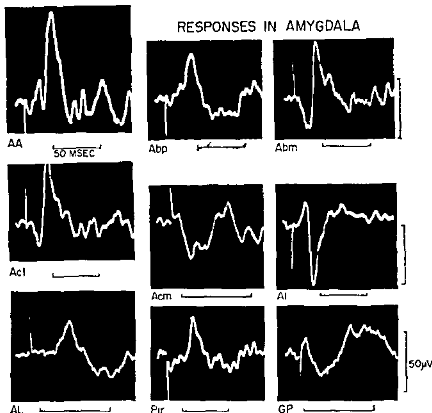


Figure 2 Responses in amygdala and adjacent areas following single shock stimulation to the hippocampus Abbreviations refer to the electrode positions as indicated in the McGill atlas of stereotaxic coordinates for the cat AA, anterior amygdala, Abp, N amygdaloideus basalis, pars parvocellularis, Abm, N amygdaloideus basalis, pars magnocellularis, Acl, N amygdaloideus centralis, pars lateralis, Acm, N amygdaloideus centralis, pars medialis, AL, ansa lenticularis, Pir, lobus piriformis, GP, globus pallidus

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a direct path from the septum to amygdala may well occur, modifications in response to monotonous stimuli or stimuli with emotional content could well take place through this cortical system.

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DISCUSSION

Harris. We have had some experience on two of the points discussed by Dr. Green. Firstly, regarding the method by which stimuli pass from the hypothalamus to the anterior pituitary by the hypophysial portal circulation; and secondly, the nervous pathways through which the sensory modality of vision influences the hypothalamus.

In regard to the first point I would like, if I may, to add a note of historic interest. As is well known, Professor G. T. Popa and Dr U Fielding were the first to describe the portal vessels of the pituitary stalk. In 1935, Popa was working in Cambridge and had with him a collection of slides showing these vessels in the human These slides he showed me and told me the story as to how these vessels were first seen It appears that Professor Rainer of Bucharest, a pathologist, first noted them on naked eye examination of the pituitary stalk in the autopsy room, and he observed they were more obvious in humans that had come to a traumatic end Rainer suggested to Popa, who was then one of his students, that he dissect out the hypothalamus, pituitary stalk, and gland in one block and cut microscopic sections to see the more detailed anatomy of the vessels of this area Popa did this and observed that the vessels began as capillaries in the tuber cinereum, passed down the stalk as large trunks to end in the sinusoids of the anterior lobe.

Some years later when Popa was visiting University College in London he happened to mention these findings to Professor Elliot Smith Elliot Smith, realizing the possible significance of such vascular connections, encouraged Popa and Fielding to investigate these vessels in more detail The outcome of this work

was the two papers by Popa and Fielding in 1930 and 1933 (Popa, G. T., and U. Fielding: *J. Anat.*, 65 88, 1930, and *J. Anat.*, 67:227, 1933).

The second point I would like to mention is with regard to the possible pathways of sensory fibers from the optic chiasma or optic tracts to the hypothalamus. We have been working recently on the light-induced estrous response of ferrets and in this field there is a lack of knowledge regarding the neural connections between the visual pathway and the hypothalamus. The only work that promises help in this direction is that of Frey from Switzerland, who describes in dogs an optico-hypothalamic tract passing from the region of the optic chiasma into the hypothalamus (Frey, E.: *Bull. schweiz. Akad. med. Wissench*, I:115, 1951). I would like to know very much, Dr. Green, if you have observed such fibers in any of the many forms you have investigated. A further question I would like to ask, also related to the nervous connections of the hypothalamus, is whether in your opinion the mammillary body gives fibers to the region of the tuber cinereum. In a previous investigation we obtained experimental data that the mammillary body was related to the secretion of ACTH, and it is therefore of interest to know what are the fiber tracts relating the mammillary body to the hypophysial region

Gloor: I would like to ask Dr Green if the observations he made during coitus were in intact animals or if he had any observations on animals without cortex; especially with leads in the diencephalon and if he obtained the same pattern of discharge as in the hypothalamic studies

Dr. D Duncan, Galveston, Texas I would like to ask a question In a paper some years ago Fabing observed a profound effect on the activity of the anterior pituitary by removal of the vidian nerve and vidian ganglion I have never seen that work confirmed or specifically denied I would like to know if Dr Green has anything to say about that The reason that remains in my mind is that it would be the logical place that a parasympathetic supply could approach the anterior lobe, since it derives from the buccal cavity.

Green In regard to Dr Harris' first question on connections

to the mammillary body, we spent some time looking for these and were unable to trace such fibers. It might be remembered that in some animals there is an infra-mammillary recess of the third ventricle which would mean that these fibers would have to take a rather long recurrent path. I do not intend to imply that because we could not find fibers from the mammillary body to the neural stalk, connections are not present. There may, of course, be relays from the mammillary body to other areas and thence to the pituitary stalk, and of course it may be possible for some other people to demonstrate these connections. All I can say is that we were never able to see any. With regard to the optico-hypothalamic fasciculus, I never studied this particularly I believe Morin has done some work on it and concluded that it was an artefact due to a peculiar method of sectioning.

In answer to Dr. Gloor, these animals were intact and unanesthetized, with implanted electrodes. We did not make any lesions in addition. It is difficult enough to get satisfactory results in intact animals during coitus since you have to take the records rather soon after you have implanted the electrodes. In two or three weeks, implanted electrodes frequently give trouble from polarization.

With regard to Dr. Duncan's question on the vidian nerve and ganglion, I think Dr Harris will be able to answer this in more detail than I The work of Dr Vogt would seem to exclude the vidian nerve as being important in the activation of the anterior pituitary.

NEUROSECRETION IN THE CENTRAL NERVOUS SYSTEM*

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THE STRUCTURAL APPEARANCE of the neuron is characterized by its stability. Fluctuations in the functional stage within physiological boundaries generally do not express themselves in morphological changes which can be detected by ordinary microscopic techniques. On the other hand, the microscopic appearance of gland cells reflects only momentary cell activity. For instance, the process of formation and discharge of glandular products in many cases dictates the characteristic—and often very different—features of gland cells The combination of such diversified properties in one single cell type, at first glance, is difficult to understand. However, certain neurons play an important role as producers of hormones, the source of which was ascribed formerly to other cells or was not known at all The term "neurosecretion" is used for such peculiar manifestations in their entirety (Scharrer, 1928, see also his extensive chapter in v. Moellendorff's *Handbook*, 1954).

Neurosecretory neurons are described in many classes of the animal kingdom from invertebrates to man. Groups of such cells are located in different parts of both the central and the peripheral nervous systems, where they act as sources of hormones of varying nature

In this paper we deal with neurosecretory cell groups in the

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(PHS B-364(c)) from the National [illegible]
ness, of the National Institutes of H[illegible]
C M Pomerat, and in part by a grant from the Deutsche Forschungsgemeinschaft

ventral anterior hypothalamus of vertebrates and man, namely, the supraoptic and paraventricular nuclei which are part of a hypothalamo-neurohypophysial neurosecretory system. The cell bodies of these neurons form closely-packed nuclei just above the optic chiasma and the origin of the optical tract and directly under the ependyma of the third ventricle below the anterior end of the sulcus limitans. The nuclei are richly vascularized. The surfaces of the pericarya are usually in very close association with blood capillaries which produce an architectural appearance peculiar to these nuclei. The axonic processes of both the supraoptic and paraventricular neurons run downward through the hypophysial stalk into the posterior lobe of the pituitary, thus forming the supraoptico-hypophysial tract. The endings of this tract form a network in the posterior pituitary with denser parts in the perivascular zones and less dense parts in the intermediate zones. In fact, the main bulk of the tissue in the posterior lobe consists of such nerve fiber endings.

It was in these hypothalamic cell groups that Scharrer (1928 and later) described inclusions similar to those in gland cells. In many cases, Scharrer and his collaborators (Palay, 1945) observed such granular inclusions not only in the pericarya but also along the axon processes. However, the ordinary staining methods applied did not demonstrate all of the granules present in this system. Bargmann (1949), applying Gomori's chrome-alum-hematoxylin method, showed that this staining method was excellent in demonstrating granular inclusions all over the neurosecretory neurons, from the pericaryon down to the endings of the axon processes in the posterior pituitary. Thus it was possible to stain selectively the neurosecretory neurons due to the intake of hematoxylin by the granular masses within the cells and their processes. This gave us a very clear picture of the "neurosecretory pathway" and at the same time a basis for experimental work to determine the physiological significance of neurosecretion in this system.

When using Gomori's technique we see that not all cells of these nuclei contain the same quantity of granules. There are cells in which the cytoplasm is almost devoid of granules, while others are completely filled up. Between these two extremes one

can observe all stages of transition, giving the impression that the cells are in different stages of secretory activity

This raises the question of granule origin. Although this problem has not yet been investigated sufficiently, most observers are of the opinion that the granules are formed at the expense of Nissl substance, because cells with few or no granules generally exhibit many Nissl bodies, whereas cells containing a large amount of granular inclusions show only very few Nissl bodies However, this requires further investigation, the mitochondria and the zone of Golgi especially have to be taken into consideration All investigators nevertheless agree that the granules originate within the pericaryon.

If one accepts this opinion, the problem is of course of the means by which the neurosecretory granules get into the axon processes and their endings in the posterior lobe of the pituitary The working hypothesis that there might be a protoplasmic flow from the cell bodies toward the axon endings by which the granules are carried into the posterior lobe had to be supported or to be refuted by experimental procedures, such as cutting through the supraoptico-hypophysial tract, e g, in the hypophysial stalk. If there were such a protoplasmic flow and if it continued only a short time after cutting the tract, one should be able to find a piling up of neurosecretory substance central to the site of transsection, whereas the peripheral part of the system, i e, the distal fiber stumps and the posterior lobe, would be expected to be emptied after a certain time Our transsection experiments confirmed this hypothesis For final substantiation, however, it was necessary to observe and to demonstrate directly such a protoplasmic flow within axon processes of this kind Since this, for optical reasons, is impossible in the living animal, the method of choice was that of tissue culture Here we can observe living cells under the microscope and in addition use time lapse cinematography Despite some theoretical doubts, it was possible to maintain neurosecretory neurons *in vitro* for long periods of time as well as to make moving picture records of peripherally directed protoplasmic movements within axon processes

Up to this point, then, we believe that a granular material, which stains selectively with chrome-alumhematoxylin, is elab-

orated in the pericarya of the supraoptic and paraventricular neurons and that it is transported by means of a protoplasmic flow from the hypothalamus via the supraoptico-hypophysial tract into the posterior pituitary, where it is stored.

The next question concerns the physiological significance of this substance. The working hypothesis concerning this point was that the stainable substance possibly had some connection with hormones thought to originate in the posterior pituitary. As anatomists we faced the fact that certain hormones can be extracted from the posterior pituitary but at the same time we find no histological evidence for a secretory nature of the posterior lobe cells. Hormone producing cells observed in true endocrine glands show distinct signs of their secretory function, i.e., granules or droplets or vacuolization and a characteristic behavior of the mitochondria and the Golgi apparatus. So far none of these characteristics have been shown definitely to be present in any cell type of the posterior lobe. However, when we consider that the main bulk of posterior lobe tissue consists of nerve fibers originating in the neurosecretory nuclei of the hypothalamus, we feel that we cannot classify the posterior lobe as a distinct anatomical and functional entity In fact, the posterior lobe is only part of a hypothalamo-hypophysial system which should be regarded as a whole. If we attribute to this system the elaboration of the so-called posterior lobe hormones, then it should take place in those parts of the system, where we see histological evidence for a secretory function, namely, the supraoptic and paraventricular neurons.

Following the above reasoning then, we endeavored to see whether there was any connection between the histologically demonstrable neurosecretory substance and the so-called posterior pituitary hormones. It is generally known that in an animal experimentally dehydrated by withholding drinking water for a certain time or by administering hypertonic salt solutions, the content of antidiuretic hormone in the posterior lobe decreases rapidly. It is of great interest that under the same experimental conditions the amount of the stainable material also decreases The behavior of the neurosecretory material under these circumstances, therefore, is the same as that of the antidiuretic hormone.

Furthermore, if the stainable secretory granules have anything to do with the so-called posterior lobe hormones it should be possible to demonstrate these hormones in any part of the hypothalamo-hypophysial system which contains neurosecretory stainable substance. We feel that we have accomplished this Employing a simple technique, one can isolate the different parts of the neurosecretory pathway. the supraoptic nuclei, the paraventricular nuclei, the supraoptico-hypophysial tract in the median eminence, the hypophysial stalk and the posterior lobe. Extracts of all these parts have been tested for their hormonal activities. Bioassays performed in the bladder fistula dog, in the decapitated cat and in the isolated uterus of the guinea pig show activities characteristic of antidiuretic, vasopressor and oxytocic hormones. Thereby, the amount of hormones found in the different parts of the neurosecretory pathway can be correlated with the amount of stainable material present. There is, for instance, a species difference. Where we find abundant amounts of neurosecretory material, as in the dog, the amount of hormones found in bioassays is high correspondingly. In other species, for instance ox or pig, with a low granule content, the hormonal activity is also low.

As already briefly indicated, the posterior pituitary is rapidly emptied of both hormones and stainable material under conditions of water balance disturbance The same is true for the cell bodies and nerve fibers in the whole neurosecretory system. It was therefore of interest to observe the reaccumulation of both substances in the entire system after reestablishment of the water balance following a period of thirst It was found that the posterior lobe in all instances was more rapidly depleted than the other parts of the system, i e, cell bodies and nerve fibers Also, the reaccumulation in the posterior lobe occurs before it does in other parts of the system We assumed that under these experimental conditions the storage organ (the posterior lobe) is emptied first and that it is refilled earlier after the stress is removed

In pursuing our theory further, another experiment was of considerable importance. First, we emptied the entire neurosecretory system of dogs, then performed a stalk transsection, following which we observed reaccumulation of hormones and

stainable substance throughout the system. As we expected, the distal fiber stumps and the posterior lobe remained empty, whereas a very abundant reaccumulation was observed above the site of transsection in the cell bodies and in the central fiber stumps. The reaccumulation in these parts was much greater than that observed in animals in the same stage of recovery but without previous dissection of the hypophysial stalk. This indicates clearly that hormones and stainable substances are elaborated in the pericarya of the neurosecretory cells. If transport into the posterior lobe is prevented, such as after stalk section, the secreted materials pile up above the site of transsection. In all these experiments quantitative measurements of hormones as well as of stainable substance were made. One-half of the entire system was used to prepare extracts for bioassays, the other half served to make histologic sections In the sections the amount of stainable substance was determined by light absorption measurement and compared with the hormone content as determined by bioassays. It was found that the variations in amounts of both substances under various experimental conditions were always parallel to each other.

The above observation led us to wonder whether the stainable granules were a hormonal substance in themselves In order to determine this it would be necessary to isolate the granules and to perform bioassays of them. It was found that the granules were easily dissolved in acetone or alcohol. If fresh tissue was put into one of these fluids or in a mixture of them, the granules went into solution. After evaporation the stainable granules could be demonstrated in the residue A suspension of the stainable material or an extract made of it and checked for hormonal activity by means of bioassays yielded no hormonal effect On the other hand, extracts of the tissue freed of granules produced the same effect as before dissolution of the granules, namely, evidence of hormonal activity This shows then that the stainable substance is not identical with the hormones but is always closely associated with them in the tissue It is possible that the stainable material is a carrier substance or vehicle for the hormones. Such a relation exists in the thyroid gland, where the active principle is embedded in an inactive colloid-like substance

The general assumption until recently was that the posterior lobe hormones are elaborated by special cells in the posterior lobe, the pituicytes But, as already mentioned, we were unable to find general histological evidence of a secretory nature for these elements. If our new concept about the hypothalamic origin of the so-called posterior lobe hormones was to be proven correct, we had finally to re-examine reports dating back some 20 years on hormone formation in explants of posterior pituitary tissue *in vitro* Furthermore, we had to find out whether it is justified to postulate on a histological basis the existence of a special type of secretory or glia cell in the posterior lobe (the "pituicyte").

In repeating the experiments of Geiling and Lewis (1935) we obtained the following results: when posterior lobe tissue derived from normal animals is explanted, the presence of hormones can be demonstrated for several days. After the sixth to seventh day of cultivation the hormone content of the cultured material decreases very rapidly. In cultures of posterior lobe derived from dehydrated animals we were unable to find any hormonal activity at any time. This evidence seemed to justify the conclusion that hormone formation in posterior lobe tissue *in vitro* does not take place. According to observations of fixed and stained cultures, as well as moving picture records of living posterior lobe glia cells *in vitro,* we are inclined to identify them with protoplasmic astrocytes, which are the supporting elements present in all regions of the central nervous system

Up to now we have been unable to find hormonal activity in explants of supraoptic and paraventricular nuclei. However, if we consider that from the total explanted amount of about 90,000 neurosecretory cells from one animal, only eighty to one hundred are able to survive *in vitro*, it may be assumed that the amounts of hormonal substances yielded by these remaining cells is too small to be detected by the relatively crude bioassay methods applied thus far. More sensitive techniques may yield more satisfactory results in the future.

These surviving neurosecretory neurons are very good test objects for observing the general behavior of living neurons *in vitro* as well as for short term experiments concerning their reaction to physiological or other stimuli We know, for instance,

that the nuclei of neurosecretory cells *in vivo* under the influence of alterations in water balance with the accompanying changes in the osmotic pressure of the blood, respond with enlargement or swelling, which is reversible when the stress is terminated. Such a "functional nuclear swelling" (Benninghoff, 1949) is regarded generally as a sign of high secretory activity in gland cells. *In vitro* we can also observe this peculiar behavior of the nuclei in *neurosecretory cells if we expose the cultures under* the microscope to a nutrient solution containing a slightly higher NaCl concentration than the normal nutrient. The higher osmotic pressure apparently is an adequate stimulus for those cells. Under the same experimental conditions the nuclei of other neurons, e g , Purkinje cells used as controls, do not respond to an increase of the salt concentration in the nutrient medium. These latter experiments point in a direction which suggests that the supraoptic and paraventricular neurons occupy a unique and peculiar position within the nervous system.

CONCLUSIONS AND SUMMARY

Considerable evidence has been presented that the antidiuretic, vasopressor and oxytocic hormones are not produced in the posterior pituitary They originate by a neurosecretory process in the neurosecretory nerve cells of the supraoptic and paraventricular nuclei in the hypothalamus and migrate within the nerve fibers of the supraoptico-hypophysial tract into the neurohypophysis Here they are stored and, if necessary, released The product of the neurosecretory cells consists of a histologically stainable carrier substance which contains the hormones and which can be separated from them by solution in various organic fluids. This concept is based upon the following observations:

(1) *The hormone content of the supraoptic and paraventricular nuclei*, and supraoptico-hypophysial tract and the neurohypophysis – as shown in bioassays – parallels in general the amount of stainable material present in histologic sections. The hypothalamo-hypophysial system can be depleted of neurosecretory substances (carrier substance and hormones) by dehydration of the experimental animals After re-establishment of water balance, a re-accumulation of both fractions is observed.

(2) After interruption of the supraoptico-hypophysial tract (neurosecretory pathway) in dehydrated animals, carrier substance and hormones accumulate in the central fiber stumps and in the nerve cells during the period of recovery, while the peripheral fiber stumps and the posterior lobe remain empty.

(3) Bioassays of posterior pituitary tissue grown *in vitro* show that the posterior lobe under these conditions also is unable to produce hormones The hormones transferred with the explants (storage tissue) into the culture medium are inactivated within seven to ten days, at which time the nerve endings have degenerated and the stainable carrier substance has disappeared. Neurosecretory cells grown *in vitro* for from twelve to sixty-eight days contain small amounts of stainable neurosecretory substance. They are nearly ideal general test objects for the study of neuronal behavior *in vitro* as well as for various experiments with living nerve cells under visual and time lapse microcinematography observation.

(4) Time lapse microcinematography (phase contrast) of axon processes of neurosecretory neurons *in vitro* (perfusion chamber technique) gives evidence of a peripherally directed transportation of substances within the axoplasm of these cells

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that the nuclei of neurosecretory cells *in vivo* under the influence of alterations in water balance with the accompanying changes in the osmotic pressure of the blood, respond with enlargement or swelling, which is reversible when the stress is terminated. Such a "functional nuclear swelling" (Benninghoff, 1949) is regarded generally as a sign of high secretory activity in gland cells. *In vitro* we can also observe this peculiar behavior of the nuclei in neurosecretory cells if we expose the cultures under the microscope to a nutrient solution containing a slightly higher NaCl concentration than the normal nutrient. The higher osmotic pressure apparently is an adequate stimulus for those cells. Under the same experimental conditions the nuclei of other neurons, e.g., Purkinje cells used as controls, do not respond to an increase of the salt concentration in the nutrient medium. These latter experiments point in a direction which suggests that the supraoptic and paraventricular neurons occupy a unique and peculiar position within the nervous system.

CONCLUSIONS AND SUMMARY

Considerable evidence has been presented that the antidiuretic, vasopressor and oxytocic hormones are not produced in the posterior pituitary. They originate by a neurosecretory process in the neurosecretory nerve cells of the supraoptic and paraventricular nuclei in the hypothalamus and migrate within the nerve fibers of the supraoptico-hypophysial tract into the neurohypophysis. Here they are stored and, if necessary, released. The product of the neurosecretory cells consists of a histologically stainable carrier substance which contains the hormones and which can be separated from them by solution in various organic fluids. This concept is based upon the following observations

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between the glia of the posterior lobe and stalk and astrocytic glial elements found in other parts of the central nervous system. Certainly I would not have expected there to be any great difference. They are both derivatives of the same general basic genetic material However, sectioned and stained preparations of pituicytes and other astrocytic glia suggest some morphological differences between these two cell types. Whether they are specific types or merely appear different because of the morphology of other elements with which they are associated in the posterior lobe remains unsettled in my mind Whether or not the pituicytes play some functional role in the process of storage and/or release of neurosecretion remains a nebulous question Perhaps Dr. Hild would comment about it.

Dr. Drager, Galveston, Texas: I would like to ask one question It has been reported by Selye, Ortmann, yourself and others that under conditions of dehydration mitoses of pituicytes have been observed Have you any explanation of this fact?

Dr. Brown, Omaha, Nebraska It is my understanding that if you section the stalk in humans and in animals, diabetes insipidus does not occur—provided the stalk section is not too low. How does that tie in with this theory of migration of substances from the hypothalamus?

Dr. Pirth, Virginia I would like to ask two questions: Have you ever seen the neurosecretory granules reappear in the distal segment after stalk section, and in dehydrated animals in which there is disappearance of secretory granules in the posterior pituitary, would injections of posterior pituitary extracts stimulate recurrence of these granules?

Dr Reichlin, St Louis, Missouri I wonder if you would comment about the relation of the neurosecretory granules to the regulation of the anterior pituitary with reference to the primary plexus of the portal circulation

Dr Halpert, Houston, Texas. I would like to ask whether any relation has been made to the actual secretion and the granules —the golden yellow granules—that are seen in the cells of the posterior hypophysis in humans, and what the magnification was in this beautiful movie

Hild, to Dr Russell The assay methods of posterior pituitary

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DISCUSSION

Dr. Russell, Galveston, Texas: There are several things that come to my mind in the course of the discussion. One of the most frequent criticisms of the present concept of neurosecretion is the problem of the direction of flow of this material. Dr. Hild, *I think, clearly answered this question I am sure that his illus*trations demonstrate that the direction of the flow of this material is down the infundibular stalk from the hypothalamus to the hypophysis, and not in the other direction as some investigators prefer to believe

There is one question which I feel might be worthwhile for Dr Hild to answer. We discussed this subject yesterday afternoon There has recently appeared some very serious criticism of the assay methods which have been used in pharmacological estimation of hormonal content in the hypothalamo-hypophysial system We in our laboratory have battled the assay problem and have come to much the same conclusions that he has, and I would like him to make the comments that he made yesterday about the methods of assay for these hormones.

I am not especially familiar with tissue culture methods and in tissue culture material I find it difficult to differentiate one glial type from another Those who have studied this problem extensively do seem to be able to make this distinction. I do know that Dr Hild has made the statement here, and elsewhere, that in tissue culture he is unable to appreciate any difference

neurosecretory granules in the peripheral fiber stumps and in the infundibular process will disappear. After prolonged time (twelve to fourteen days) all nervous tissue distal to the site of transsection will degenerate. No re-accumulation of granules has been observed at any time in the distal segment.

Concerning your second question, we have not done any experiments. It may be possible that the injection of posterior pituitary extracts into dehydrated animals could unburden the neurosecretory system (substitution therapy) and that in this case neurosecretory granules could reappear.

To Dr. Reichlin· This is a very interesting question which Dr. Harris probably can answer more satisfactorily. Histologically we can see that (especially clear in the seal) many neurosecretory fibers end around the primary loops of the portal vessels, but I have no explanation of the physiological significance of this fact

To Dr. Halpert There exists no relation between secretory granules and those pigment granules that are often seen in the posterior hypophysis in humans. The later ones often contain iron which can be detected by the Turnbull reaction In unstained sections this pigment appears as a brown granular material

The original magnification of the movie is 200X to 400X on the film (different in different sequences). Beyond this, the enlargement by projection on the screen in this room is, of course, very much higher

To Dr Brown· The occurrence of diabetes insipidus after stalk section depends much upon the site of transsection. If the transsection is "low" (very close to the infundibular process) diabetes insipidus does not necessarily occur. After "high" transsection (very close to the hypothalamus) diabetes insipidus appears regularly Not all axons of the secretory neurons reach the infundibular process, many of them terminate already in different levels of the stalk. Therefore, after low transsection a considerable number of secretory neurons remains completely uninjured and their activity is apparently sufficient to maintain a normal water balance If after high transsection almost all

hormones are very numerous. It depends upon the experience of the experimentalist which method he prefers. A method which works well in the hands of a particular investigator is sometimes criticized harshly because its results are more inaccurate in another investigator's hands. One example only. In the original investigation, together with Dr. Zetler (Pharmacological Institute, University of Kiel), we assayed the oxytocic activity with isolated uteri of mice of a certain strain, using them at a certain time of the genital cycle. The accuracy of the test largely depends upon the equality of the initial spontaneous uterine contractions. Repeating this method in Galveston it proved to be quite inadequate because the uteri of the mice here available did not exhibit such equal spontaneous contractions. As long as we do not have bioassay methods which give reproducible results in every worker's hands, we should not worry too much about occasional criticisms

Regarding the comments about "pituicytes"· I am very much inclined to abandon the term pituicyte There are no significant differences between the glia of the posterior lobe and astrocytic glia in any other parts of the central nervous system It does not matter from what point of view you look at the problem or whether you use fixed and stained sections or the tissue culture method. Morphologically, the glia of the posterior pituitary consists of protoplasmic astrocytes. It seems only logical that they could have a function in the mechanism of releasing neurosecretory substances from the nerve endings (neuroplasm) into the blood stream. This possibility is in accordance with our concepts of glia cell function in general (link between circulatory system and nervous substance) Certainly they do not have a secretory function The particular cells in the posterior lobe of the rat lately much emphasized by Drager, Russell and Rennels are not necessarily glial elements

Concerning the distinction of glial elements *in vitro*, to anyone observing living glia *in vitro* it is very easy to distinguish with certainty at least the following forms protoplasmic and fibrous astrocytes and oligodendroglia All these types show quite characteristic shape, structure, and movements

To Dr Pirth After transsection of the hypophysial stalk the

HYPOTHALAMIC CONTROL OF THE ANTERIOR LOBE OF THE HYPOPHYSIS

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THERE CAN be little doubt that the hypothalamus is largely responsible for maintaining and regulating the activity of the anterior pituitary gland Marshall (1936, 1942, 1955) was one of the first to draw attention to the role played by stimuli arising in the external environment in the control of the breeding season and estrous cycle. He summarized his views as follows.

> " . and it would appear certain that many external factors which regulate the cycle act through the intermediation of the central nervous system upon the anterior pituitary, this gland playing the part of a liaison organ between the nervous system which is affected by stimuli from without and the endocrine system . . "
>
> (MARSHALL, 1942)

Much evidence has accumulated that the central nervous system in general, and the hypothalamus in particular, are intimately concerned with the secretion of the gonadotrophic, adrenocorticotrophic (ACTH) and thyrotrophic (TSH) hormones The well known effects of emotional stress stimuli in causing discharge of ACTH, and the effects of such stimuli in causing inhibition of the thyroid gland, would suggest the participation of the hypothalamus in regulating the secretion of these trophic hormones.

In the following account, a brief review will be given of the evidence relating the hypophysial portal vessels to the hypothalamo-hypophysial mechanism. This will be followed by a summary of recent investigations in our laboratory on the regulation of secretion of ACTH, TSH, and the follicle-stimulating hormone (FSH), and from this and other data a general view will be pro-

neurosecretory fibers are sectioned, their cell bodies will undergo retrograde degeneration and diabetes insipidus is the result.

To Dr. Drager: Mitoses of posterior lobe cells under conditions of dehydration have been observed by several authors, but much more work has to be done in order to find an explanation of this fact. I have seen such cell divisions in dehydrated frogs and rats. On the other hand, in over one hundred dehydrated dogs I have not seen one mitosis in the posterior lobe. I feel uncertain as to the nature of such dividing cells How can we be sure that they are "pituicytes," since all cell types during mitosis round up and look alike in the stained preparation?

Dr. Guillemin: May I make just one comment regarding these "mitoses" in the cells of the posterior lobe of the pituitary. In some of the above-mentioned experiments by Selye, 20 cc of five per cent or fifteen per cent sodium chloride were injected intra-peritoneally in 150 to 250 gm rats, that is to say that these conditions were highly abnormal. Figures resembling mitoses, rather than true mitoses, were seen practically in all the nervous system, including cells of the brain cortex, Purkinje and others and in the adrenal medulla. As Dr. Hild concluded, the significance of these mitotic figures seems very doubtful

HYPOTHALAMIC CONTROL OF THE ANTERIOR LOBE OF THE HYPOPHYSIS

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There can be little doubt that the hypothalamus is largely responsible for maintaining and regulating the activity of the anterior pituitary gland Marshall (1936, 1942, 1955) was one of the first to draw attention to the role played by stimuli arising in the external environment in the control of the breeding season and estrous cycle. He summarized his views as follows.

> "... and it would appear certain that many external factors which regulate the cycle act through the intermediation of the central nervous system upon the anterior pituitary, this gland playing the part of a liaison organ between the nervous system which is affected by stimuli from without and the endocrine system . ."
>
> (Marshall, 1942)

Much evidence has accumulated that the central nervous system in general, and the hypothalamus in particular, are intimately concerned with the secretion of the gonadotrophic, adrenocorticotrophic (ACTH) and thyrotrophic (TSH) hormones The well known effects of emotional stress stimuli in causing discharge of ACTH, and the effects of such stimuli in causing inhibition of the thyroid gland, would suggest the participation of the hypothalamus in regulating the secretion of these trophic hormones

In the following account, a brief review will be given of the evidence relating the hypophysial portal vessels to the hypothalamo-hypophysial mechanism This will be followed by a summary of recent investigations in our laboratory on the regulation of secretion of ACTH, TSH, and the follicle-stimulating hormone (FSH), and from this and other data a general view will be pro-

posed of the relation of the nervous system to the activity of the anterior pituitary and its target glands.

EVIDENCE RELATED TO THE FUNCTION OF THE HYPOPHYSIAL PORTAL VESSELS

As discussed above, in the article by Dr. J. D. Green, the anatomical link by which the hypothalamus regulates the adenohypophysis has long been a subject of discussion. The great paucity of nerve fibers in this part of the gland made it seem unlikely that the hypothalamic nerve control was mediated by a direct nerve supply. When it was found that the tuber cinereum of the hypothalamus is constantly connected (in all forms from amphibians to man) to the anterior pituitary gland by a portal system of blood vessels, and that the blood flows in these vessels *from* the primary plexus in the median eminence of the tuber cinereum, through the large vascular trunks of the pituitary stalk, *to* the sinusoids of the anterior pituitary, the suggestion was put forward that neural control of the gland was mediated via these vessels

(1) *Electrical stimulation.* Stimulation of the hypothalamus and pituitary with observation of any resulting discharge of anterior pituitary hormones has now been performed by several groups of workers Markee, Sawyer, and Hollinshead (1946) and Harris (1948) both reported that discharge of gonadotrophic hormone in the rabbit could be elicited by stimulation of the hypothalamus but not by stimulation of the pituitary gland directly. Similarly de Groot and Harris (1950) found that electrical stimulation of the posterior region of the tuber cinereum or mammillary body of the rabbit resulted in discharge of ACTH as revealed by a lymphopenic response, but that stimulation of the pituitary gland directly failed to show any sign of hormone secretion Discharge of ACTH has also been observed to follow hypothalamic stimulation by Hume and Wittenstein (1950), Hume (1953) and Porter (1954)

One explanation why electrical stimulation of the hypothalamus may activate the anterior pituitary whereas direct stimulation of the gland does not is that the gland is normally excited humorally If hypothalamic nerve fibers liberate some chemical mediator into the hypophysial portal vessels, and this sub-

stance is thereby carried to the gland cells to excite or inhibit their activity, it would be reasonable to suppose that electrical stimuli applied to the pituitary stalk or gland would be ineffective (the humoral part of the pathway not being open to electrical excitation).

(2) *Pituitary stalk section* In the literature of 10 to 20 years ago there are many accounts of experiments in which anterior pituitary activity was studied following section of the pituitary stalk, but the results obtained by the different workers were highly discordant (see Harris, 1955) One explanation for the discrepancies of these findings became apparent when it was found that the hypophysial portal vessels have marked powers of rapid regeneration after simple, clean resection of the pituitary stalk (Harris, 1950) In this latter study it was also found possible to correlate the return of reproductive activity (and therefore gonadotrophic secretion) with portal vessel regeneration. In animals in which such regeneration was prevented by the insertion of plates of paper between the cut ends of the stalk, a state of anestrus supervened and at autopsy the reproductive organs were found to be atrophic This study indicated the importance of an intact or regenerated portal vascular system for gonadotrophic secretion in the rat A similar correlation has been drawn by de Groot (1952) in regard to the secretion of ACTH in the mouse

(3) *Anterior pituitary transplantation* Many studies have revealed that transplantation of the adenohypophysis to a site situated at a distance from the sella turcica results in a marked diminution in anterior or pituitary activity. The pars distalis stands in marked contrast to other endocrine organs, such as the ovary, testis, thyroid and adrenal cortex, since they may be transplanted and in a high proportion of cases have been found to function in a relatively normal manner On the grounds that the latter group of glands would receive their physiological stimulus (the respective pituitary trophic hormones) wherever they were placed in the systemic circulation but the anterior pituitary would receive its stimulus only if placed in the vascular field of the hypophysial portal vessels, an investigation was undertaken (Harris and Jacobsohn, 1952) to see whether anterior pituitary

transplants, placed at a site where they might become revascularized by the portal vessels, would show functional activity.

In control experiments similar transplants were placed under the temporal lobe of the brain. It was found that. (a) anterior pituitary tissue placed outside the sella turcica but in the subarachnoid space under the cut pituitary stalk became revascularized in a large proportion of cases by the hypophysial portal system; (b) in many instances anterior pituitary tissue revascularized by this system showed apparently normal function; (c) in the control experiments the pituitary tissue grafted and "took" well, acquiring a rich vascularization, but showed little if any functional activity; (d) pituitary tissue taken from male donors might maintain normal female reproduction functions if transplanted under the pituitary stalk of a female recipient, and (e) pituitary tissue obtained from newborn animals would show precocious development and maintain adult gonadotrophic functions if transplanted in this way into an adult host. The conclusion was drawn that anterior pituitary tissue was plastic in nature and that it depended upon the vascular connection with the hypothalamus for the maintenance and the pattern of its functional state

ACTIVITY OF "DENERVATED" PITUITARY TISSUE

Since many endocrine glands show what may be called autonomous activity when deprived of their excitatory stimulus (cf adreno-cortical and thyroid activity after hypophysectomy) it seemed of importance to study the functional capacity of anterior pituitary tissue deprived of central nervous control. The activity of the anterior pituitary isolated from neural influence may be studied *in vivo* either after pituitary stalk section, or after transplantation of the gland to a site remote from the sella turcica. The stalk section method suffers from the disadvantage that it is technically difficult to sever the stalk and to make certain that regeneration of the hypophysial portal vessels is completely prevented, whilst the transplantation technique gives poor results unless autotransplants, or transplants between closely related individuals, are used. In an attempt to answer the following questions, "(1) What is the basic level of secretion of the

'isolated' pituitary gland under constant optimum conditions? and (2) Does such a gland react to changes in the environment as does the normal gland?" a study has been undertaken of the secretion of the anterior pituitary gland of the rabbit in which the stalk has been severed and a large waxed paper plate inserted between the cut ends of the stalk.

(1) *Secretion of ACTH by the "denervated" anterior pituitary gland.* Fortier, Harris, and McDonald (unpublished) have recently studied the effect of pituitary stalk section on the secretion of ACTH in the rabbit. In these experiments the stalk was transected by a temporal approach and a large plate inserted to cover the whole upper surface of the gland Postmortem studies of these animals revealed that only slight atrophy of the anterior pituitary followed this procedure, but that the gland was still richly vascularized by systemic vessels The marked adrenal atrophy observed in these experiments indicates that the basic resting secretion of ACTH was much reduced by the operative procedure of stalk section The effect of various noxious or stressful stimuli was also studied, using the lymphopenic and adrenal ascorbic acid methods of assessing increased ACTH secretion. The results were obtained that stress stimuli which might be surmised to act via the central nervous system (neural stresses—emotional stresses induced by restraint, exposure to a cold environment) no longer caused a lymphopenia, whereas stress stimuli which probably resulted in a change in systemic blood composition or metabolic disturbance (systemic stress — surgical trauma or injection of large doses of adrenalin) still caused a lymphopenia and reduction of adrenal ascorbic acid

The conclusions were drawn that the hypothalamus acting through the pituitary stalk is largely responsible for maintaining the resting level of ACTH secretion, that the "denervated" gland still liberates small amounts of ACTH and this minor level of activity can no longer be affected by neural stresses though systemic stresses, acting via a change in the composition of systemic blood, are still able to excite an increased discharge of ACTH This conclusion is substantiated by the further finding that electrolytic lesions which destroy the median eminence produce

similar effects, in this respect, to those seen after stalk section (Fortier, Harris, and McDonald, unpublished).

In comparing the above findings with those of other workers, it is to be noted that the adrenal atrophy observed to follow stalk section is in agreement with the atrophy seen after transplantation of the pituitary gland to the eye (Cheng, Sayers, Goodman, and Swinyard, 1949; Fortier, 1951) or temporal region of the cerebral cortex (Harris and Jacobsohn, 1952). The finding of Keller, Lynch, Batsel, Witt, and Galvin (1954) that ventral hypothalamectomy does not result in adrenal atrophy in the dog is perhaps of little significance in view of the minor changes in adrenal weight that these workers observed to follow hypophysectomy in the dog.

The differential response of the "denervated" pituitary gland to different types of stress stimuli was first reported by Fortier (1951). This earlier work, in which it was shown that ocular pituitary transplants in hypophysectomized rats fail to respond to neurotropic or neural types of stress but still respond to systemic stresses, has been confirmed by the present studies. The claim that the release of ACTH in response to surgical trauma is abolished by hypothalamic lesions (Hume, 1953, McCann, 1953, Porter, 1953) is difficult to reconcile with the present view A possible explanation may be found in a decreased sensitivity of the adrenal cortex to ACTH following lesions of the median eminence and some recent findings (Fortier, Harris, and McDonald, unpublished) would support this view The fact that surgical trauma may still stimulate ACTH release in dogs in which the ventral hypothalamus has been removed (Keller, *et al.*, 1954 is in accordance with the results obtained in pituitary stalk-cut rabbits

(2) *Secretion of TSH by the "denervated anterior pituitary" gland* Brown-Grant, Harris, and Reichlin (unpublished) have investigated the effect of pituitary stalk section on the activity of the thyroid gland in the rabbit, and on the response of the thyroid to stress stimuli Changes in the level of thyroid function were observed by a method utilizing I^{131}, described by Brown-Grant, von Euler, Harris, and Reichlin (1954). In the normal rabbit it was observed that a wide variety of stress stimuli

result in a temporary (few days) inhibition of thyroid activity After pituitary stalk section and the insertion of a paper plate between the hypothalamus and the pituitary gland, the following results were obtained: (1) the activity of the thyroid gland was markedly reduced, though not to the same extent as seen after hypophysectomy, (2) the thyroid inhibition that normally follows restraint or injection of stilboestrol was abolished, and (3) the inhibition that normally follows laparotomy or injection of thyroxine was unaffected. These results are again compatible with the view that the "denervated" gland retains a residual secretion, in this case thyrotrophic, which is no longer affected by stimuli acting through the nervous system but may still be inhibited by systemic stresses.

These findings are in accord with those of Ganong, Fredrickson and Hume (1954) that lesions in, or just above, the anterior end of the median eminence may reduce the thyroidal uptake of I^{131} to the levels found in hypophysectomized animals The work of Greer (1952) and Bogdanove and Halmi (1953) which showed that hypothalamic lesions between the ventromedian and suprachiasmatic nuclei prevent the thyroid hypertrophy which normally follows administration of thiouracil, suggests that changes in the blood concentration of thyroxine affect the rate of release of TSH through the mediation of the hypothalamus The discrepancy between these findings and those seen after administration of thyroxine to stalk-cut rabbits may possibly be explained on the grounds that changes in the blood concentration of thyroid hormone may act at both a hypothalamic and anterior pituitary level, but that the neural mechanism is the more sensitive to slower and minor changes in thyroxine concentration

(3) *Secretion of FSH by the "denervated anterior pituitary" gland.* It is well known that estrus may be induced in the sexually quiescent female ferret by artificially increasing the length of day during the winter months, and it has also been established that this response is due to the extra illumination acting through the eyes and optic nerves and some unknown reflex path to stimulate the secretion of FSH from the anterior pituitary gland In order to see whether the pituitary stalk formed part of this path, Thomson and Zuckerman (1953, 1954) severed the pituitary stalk

in 17 ferrets by a buccal approach and exposed them to extra light in the winter. They believe that two of these ferrets, which reacted to light exposure by becoming estrus, did so in the absence of any vascular connections between the median eminence and adenohypophysis and concluded that the hypophysial portal vessels do not form part of the pathway by which light stimulates anterior pituitary secretion in the ferret.

However, this work has been repeated by Donovan and Harris (1954) with different results. In twenty-four animals, the pituitary stalk was exposed by a temporal approach; in four, the stalk was left intact, in six, simple stalk section was performed, and in fourteen, the stalk was cut and a waxed paper plate inserted between the hypothalamus and pituitary gland. Twelve of the twenty-four operated animals became estrus following prolonged illumination during the winter months. After killing, the vascular system was perfused with India ink and serial sections cut at 160μ through a block of decalcified tissue containing the base of the skull, pituitary gland and hypothalamus.

Microscopic examination of the pituitary region revealed that. (1) the pituitary stalk had been successfully sectioned in all but the blank operated animals, (2) vascular connections between the median eminence and adenohypophysis existed in all animals that developed estrus These connections either passed across the site of a simple stalk section or around the borders of plates misplaced in position, and (3) some atrophy of the pituitary gland occurred in all animals in which the pituitary stalk was cut but the degree of atrophy in individual animals could not be correlated with the absence or presence of an estrous response The conclusion was drawn that the hypophysial portal vessels form part of the neuro-endocrine reflex path underlying the light-induced estrous response of the ferret

The difference in the observations and conclusions of Thomson and Zuckerman, and Donovan and Harris raises a point of general importance in this type of experiment In studying the effect of pituitary stalk section on anterior pituitary function, it is necessary to control the possibility of hypophysial portal vessel regeneration across the site of stalk section Such a procedure requires attention to the following points of technique

(a) The operative approach The temporal approach gives a wide exposure of the pituitary region, allows deliberate and precise transection of the stalk, permits the placement of large plates between the stalk ends and leaves the region of the stalk surrounded by undamaged tissue The parapharyngeal and buccal approaches on the other hand lack the above advantages and in particular have the drawback of producing anatomical disorganization of the region to be subjected, postmortem, to microscopic study. The examination of serial sections through the region is gravely hindered by the trephine hole in the base of the skull, by the frequent prolapse of the anterior pituitary into this bony aperture, by the bone dust introduced into the subarachnoid space, and by the fibrosis that occurs around the operative site

(b) Histological techniques In order to study the vascular anatomy of the region, it is advisable to perfuse the vascular systems of the experimental animals with some colored material, such as India ink, immediately after death Following fixation, dehydration and decalcification, a block of tissue containing the base of the skull, pituitary and hypothalamic region should be embedded in celloidin and serial sections cut at a thickness of 100-200μ Such thick sections are necessary for visualization of the vessels of supply to the anterior pituitary gland.

In view of such considerations Donovan and Harris (1954) suggested that Thomson and Zuckerman (1953), who performed stalk section by a buccal approach and who checked the anatomy of the region by serial sections cut at a thickness of 10μ, might have been mistaken in their interpretation of the results obtained in two ferrets It was thought probable that the two animals, on which the conclusions of Thomson and Zuckerman were based, might have possessed regenerated vessels passing from the median eminence to the pars distalis of the pituitary that escaped detection owing to the difficulties inherent in the techniques used These comments were apparently misconstrued by Thomson and Zuckerman (1955) who argued that a few animals that respond to extra illumination in the absence of such vascular connections are of more significance than many animals showing a "negative result" Such a philosophic view is unassail-

able, though the point of issue is still whether these two, or now three, critical animals possessed, or did not possess, regeneration of the portal vessels—rather than the significance of the results assuming the absence of such regeneration.

Recently Abrams, Marshall and Thomson (1954) have reported that cervical sympathectomy is effective in preventing the light-induced estrous response in the ferret. No evidence is given, however, that this experiment affords more than a demonstration that cervical sympathectomy results in constriction of the pupils of the eyes.

GENERAL VIEW OF THE RELATIONSHIP OF THE CENTRAL NERVOUS SYSTEM TO ANTERIOR PITUITARY ACTIVITY

From the information available at the moment, the following propositions might be taken as hypotheses to be tested by future work.

1. The anterior pituitary gland deprived of neural control shows slight autonomous activity with regard to the secretion of ACTH and TSH. The secretion of FSH seems to cease entirely under these conditions In such a respect the anterior pituitary may be compared to the thyroid gland and adrenal cortex which still function, though at a low level, after hypophysectomy

2. The functional state of the adenohypophysis appears normally to be modified by neural factors related particularly to stimuli arising in the external environment, and by systemic factors related to changes in the composition of the general systemic blood (internal environment).

3. Neural control is mediated via the hypothalamus and the hypophysial portal vessels of the pituitary stalk The most likely view at the moment is that the hypophysial portal vessels are concerned with the transmission of a humoral mediator, liberated by hypothalamic nerve fibers, to the gland

4. In a general way, the portal vessels of the pituitary stalk may be described as the anatomical connecting link between the external environment on the one hand, and the anterior pituitary and its target organs on the other The loss of activity and atrophy (partial or complete) of the adrenal cortex, thyroid and gonads

that follows "denervation" of the anterior pituitary gland may be explained in terms of loss of the effect of the multitudinous stimuli to which the organism is continuously subjected from its surroundings.

5 Although the endocrine activity of the adrenal cortex, gonads and thyroid is evident before birth (see Jost, 1953), it is only after birth—when the organism becomes exposed to a varying external environment—that nervous reflexes may modify the functional state of these glands. It may be surmised that maturation of the central nervous system allows a variable external environment to influence the secretion of ACTH about two weeks after birth in the rat (Jailer, 1950), and the secretion of gonadotrophic hormone at the time of puberty.

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DISCUSSION

Hild I have a question concerning the connections of the capillary loops of the portal system with the nervous system We know that not all fibers of the supraoptico-hypophysial tract reach the infundibular process A considerable number of them terminates already in different levels of the stalk and apparently they end mostly around the capillary loops in which area we find often fairly large accumulations of neurosecretory substance. Could it be possible that neurosecretion influences, via this vascular connection, the functioning of the anterior pituitary?

Harris It is a very interesting speculation. The idea of a neurosecretory mechanism with regard to the posterior pituitary gland is of great interest to us since we have postulated that a similar type of mechanism is concerned with the control of the anterior pituitary gland. The suggestion has of course been put forward by several workers that the Gomori stainable material of the supraoptico-hypophysial tract is liberated into the primary plexus of the hypophysial portal vessels and is carried by these vessels to influence the activity of the pars distalis. One of the difficulties encountered by this theory is that stimulation of the supraoptico-hypophysial tract electrically is not necessarily followed by any change in the rate of secretion of gonadotrophic hormone or ACTH, whereas an obvious change in such secretions can be seen to follow stimulation of the posterior part of the tuber cinereum—that is at a site removed from the supraoptico-hypophysial system On the other hand, I believe that Benoit and Assenmacher* (1953) have suggested that neurosecretory material may be concerned in the control of the anterior lobe of the pituitary, but derived from nerve fibers other than those in the supraoptico-hypophysial tract So far as I know at the moment, this is a matter of speculation

Dr Leo Kraintz I was curious to know how you eliminate restraint factors from your animals while you are measuring their iodine uptake They looked quite restrained.

Harris We believe that the amount of restraint inherent in our method of measuring the thyroidal I^{131} is not sufficient to affect the thyroid activity of the rabbits The reasons for this belief are as follows firstly, the curves representing the output of I^{131} by the thyroid gland were smooth and there was no correlation between the slope of the curve over any particular period and the frequency or grouping of the neck counts during the same period Secondly, as far as can be judged by their behavior, rabbits quickly become accustomed to the procedures involved in making neck counts Thirdly, such emotional stress stimuli as immobilization must be conducted for at least several hours before a detectable change in the rate of I^{131} output occurs We

* Benoit, J., and I Assenmacher Rapport entre la stimulation sexuelle préhypophysaire et la neurosécretion chez l'oiseau *Arch Anat micr*, 42 334-386, 1953

also noticed that rabbits subjected to the same restraint stimulus for periods of twenty-four to forty-eight hours, on several separate occasions, would "adapt" to this procedure so that the thyroidal inhibition became less obvious and might be lost.

Guillemin: Regarding the weight of the ovaries of the stalk-sectioned ferrets: there is indeed very considerable discrepancy in the weight of the gonads in the estrous animal and the successfully stalk-sectioned animal exposed to light. Is there such a difference in the weight of gonads of the stalk-sectioned non-estrous animal exposed or not to light and the weight of the gonads of the normal non-estrous animal (not exposed to light)? What I have in mind is "is there a difference in basal maintenance secretion of FSH?"

Harris. We have little data from our work on ferrets with which to answer your question We have few observations on the ovaries of normal ferrets not exposed to extra light during winter, or of hypophysectomized ferrets All we can say is that following permanent interruption of the pituitary stalk in the ferret the ovary shows very marked diminution in weight, an absence of antral follicles, and no signs of activation Similar results to these have been obtained in rats and rabbits in which stalk section has resulted in ovaries similar to those of hypophysectomized animals.

Guillemin. Would you consider the difference in the weights of the thyroids and adrenals statistically significantly different in these three groups of animals, namely: stalk-sectioned, hypophysectomized and control, in conditions which do not affect thyroid and adrenal activities?

Harris. Is your question whether the thyroid and adrenal weights of the stalk-sectioned animals are statistically different from those of the hypophysectomized control animals?

Guillemin Yes

Harris. I cannot answer that accurately at the moment. My impression is, so far as the adrenal gland is concerned, that they are markedly different. The reason I am unable to answer your question precisely is that the histological study of the pituitary glands of the stalk-sectioned rabbits has not yet been completed At the moment all operated animals are grouped under the head-

ing of "stalk-sectioned," but it is possible that some of these animals will have to be extracted from the group if we find that regeneration of the portal vessels has occurred. However, my impression is quite strong that the adrenal weights of stalk-sectioned animals will be found to be somewhat greater than the hypophysectomized rabbits, and that the thyroid activity as estimated by the radio-iodine release test will also be found to be somewhat higher in the completely stalk-sectioned animal than in the hypophysectomized.

HYPOTHALAMIC-HYPOPHYSIAL INTERRELATIONSHIPS IN THE PRODUCTION OF PITUITARY HORMONES IN VITRO

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EXPLANATION OF THE intimate mechanisms involved in the release of ACTH by the anterior lobe of the pituitary has been sought so far, only by *in vivo* experiments. Since the early observations relating the central nervous system to the stimulation of the pituitary (Bisonnette, 1931, Benoit, 1936, Marshall, 1936, Long, 1952, Markee, *et al.*, 1946), experiments with more involved techniques (Harris, 1948, Fortier, 1951, Hume, 1949, McCann, 1953) have suggested that the hypothalamic nuclei of the diencephalon constitute the upper integrative mechanism in the physiology of the anterior lobe of the pituitary or at least in some aspects of it The particular anatomy of the various formations connecting hypothalamus and adeno-hypophysis made it likely that the hypothalamic control of the pituitary should be humoral in nature, as opposed to a purely neural mechanism (Green, 1951, Green and Harris, 1947). A rich experimental evidence is in agreement with this hypothesis (Harris, 1948, Hume, 1949, McCann, 1953, Cheng, *et al*, 1949, Benoit and Assenmacher, 1953) Its value, however, would only be proven by isolation of the hypothetical hypothalamic neurohumor (s) postulated, the demonstration of their precise hypothalamic origin and the confirmation of their specific role (s) in the function of the anterior lobe of the pituitary The difficulties encountered in approaching the various questions of this problem by the classical *in vivo* experiments are numerous for reasons of techniques, they

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are perhaps unsolvable for reasons of logic (Cf. the theory of a multiple control of the anterior pituitary where non-specific systemic stress would activate the pituitary through means exclusive of the hypothalamus, Fortier, 1951).

It appeared therefore of interest to investigate simple systems where, by *in vitro* survival of tissues or organs, the experimentator could eliminate the non-specific systemic components of the reactions leading to activation of the pituitary. Having thus an approach to study the specific hypothalamic control of the pituitary, the understanding of this mechanism would make it easier to confirm or infirm its exclusivity in the *in vivo* regulation of the anterior pituitary The possibility of using *tissue cultures* techniques to study the hypothalamic control of the anterior lobe of the pituitary was suggested to us by two observations made in the department of Dr Charles Pomerat at the University of Texas Medical Branch in Galveston by one of his graduate students, Barry Rosenberg, who was to work later in our own laboratory on some of the experiments reported here.

It was noticed that the gonadotrophic activity, found in the tissue cultures fluids of the anterior pituitary, decreased very rapidly as the age of the cultures increased, in spite of excellent growth of the pituitary explants Furthermore, when thyroidectomy cells, adrenalectomy cells, or castration cells were produced in the rat by the appropriate surgical operation, these characteristic formations could not be maintained in tissue cultures of the corresponding pituitary, the normal histological picture of a normal pituitary in culture was found

The literature regarding possible secretions of hormones by tissue cultures of the pituitary, though highly conflicting (Gaillard, 1948, Martinovitch, 1953; Cutting and Lewis, 1938, Anderson and Haymaker, 1935), showed us that whenever hormonal production had been found at all, it had always been early (up to four or five days) after explantation of the tissues All this suggested that the presence of a factor (of hypothalamic origin) might be necessary to insure the proper function of growing differentiated hypophysial tissue *in vitro* and also to maintain the various "-ectomy cells" of the anterior lobe of the pituitary *in vivo* as well as *in vitro* We therefore decided (1) to study the release

of one hormone (ACTH) by explants of the pituitary in cultures, as a function of time, and (2) to investigate how this ACTH secretion could be modified by adding to the cultures of the pituitary fragments of hypothalamic tissue or hypothalamic extracts. A simple "direct" assay procedure for hypothalamic hypophysiotropic activity was contemplated along the same lines should any specific effect due to the hypothalamic tissues be demonstrated.

METHODS

(1) *Tissue Cultures.* Cultures of anterior lobe of the pituitary and various other tissues (from rat or dog) were made according to the classical roller tube method (Pomerat, 1951). Small fragments of the tissues were placed on a pyrex cover slip in a drop of clotted plasma, the cover slip being introduced with 2 cc of nutrient fluid in a pyrex test tube, then placed in a rotating drum at 37 to 38 degrees centigrade. The fluid used was one of the classical media utilized in tissue cultures, made up of human ascitic fluid fifty per cent, chicken embryonic extract (EE20) five per cent, and Earle's solution forty-five per cent. The fluids in the culture tubes were removed and changed every fourth or fifth day until the end of the experiments. Pituitary tissue was thus grown *in vitro* up to thirty-five days When hypothalamic or some other control tissues (spleen, liver, brain cortex) were cultured in combination with the pituitary, they were introduced for four days in the culture tubes of the pituitary on a similar cover slide (as described above) which was slipped back to back to the one holding the pituitary explants.

(2) *Organ Cultures* We also used the same roller tube technique for the *in vitro* survival of the whole anterior lobe of the pituitary of the rat The head of the donor animal was severed by decapitation, the pituitary gland after removal with forceps was placed on a ground glass slide in one drop of ascitic fluid for rapid separation of posterior lobe from anterior lobe with two fine blades The whole anterior lobe of the rat pituitary was then kept *in vitro* with the technique described above The fluids were changed every forty-eight hours up to eight days in several experiments of various timing designs Aqueous homogenates and saline extracts of posterior or anterior hypothalamus of bovine

origin, prepared by Dr. Walter Hearn of our Biochemistry Department, were added to the pituitary in organ culture. Homogenates of brain cortex (ox) were used as control. Pitressin (Parke, Davis & Co.) and purified vasopressin (kindly given by Dr. Vincent du Vigneaud) were similarly tested for ACTH-releasing activity.

ACTH activity in the various fluids was assayed in a purely qualitative manner in hypophysectomized rats using the adrenal ascorbic acid depletion method of Sayers, *et al.* (1948).

RESULTS AND DISCUSSION

Our dynamic study of the ACTH release by the pituitary tissues shows that ACTH activity is never found after more than four days of *in vitro* life (Table 1), thus, in spite of the fact that the explanted fragments of the pituitary show excellent outgrowth with progressive differentiation from the young fibroblastlike undifferentiated elements to the well characterized adult pituitary cells (Figures 1, 2, and 3). Now, when fragments of the posterior hypothalamus or of the median eminence are introduced and cultured jointly with the pituitary tissues from day fifteen to nineteen or twenty-two to twenty-six, ACTH activity is

TABLE 1

ACTH ACTIVITY IN FLUIDS OF TISSUE CULTURES OF THE ANTERIOR LOBE OF THE PITUITARY*

Age of Cultures Fluids Tested	*Variation in Ascorbic Acid Concentration γ/100 mg Adrenal*	*Degree of Statistical Significance*
4 days‡	−65 ± 18	p ≤ 0.02
4 days†	−101 ± 18	p < 0.01
8 days‡	+22 ± 22	
12 days†	+23 ± 20	
15 days‡	−14 ± 5	p = 0.05
19 days‡	−10 ± 11	p > 0.05
19 days†	+38 ± 22	
26 days‡	+61 ± 9	
31 days‡	+66 ± 25	

‡ Dog tissues

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* Six to eight hypophysectomized rats per assay. Figures indicate arithmetic means and standard error. Statistical significance related to initial adrenal ascorbic acid level in Sayers test. Footnote applies also to Table 2

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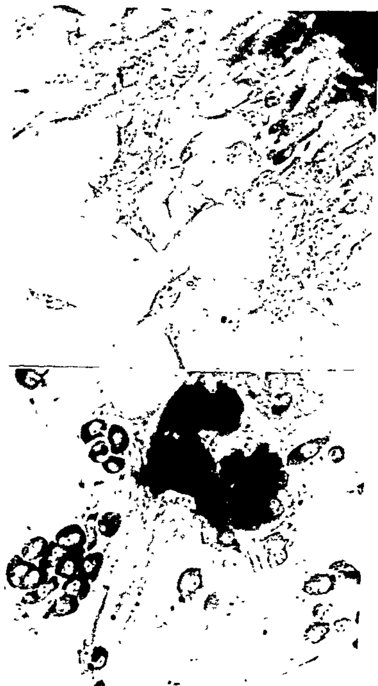


Figure 1 Young undifferentiated cells of the outgrowth of a six-day old tissue culture of the anterior pituitary (Azan, x250)

Figure 2 Numerous differentiated cells in the outgrowth of a fifteen-day old tissue culture of anterior pituitary (Azan,

found anew in these same cultures of the pituitary which had ceased to release ACTH by themselves two or three weeks beforehand (Table 2). There is no ACTH activity in the fluids of the cultures of the hypothalamic tissues. There is no ACTH released by the pituitary cultures when brain cortex, spleen, liver, or anterior hypothalamus are placed in combined cultures with the anterior pituitary (Table 2).

Such results seem to prove beyond doubt that an entirely humoral influence of the hypothalamus on the release of a hormone by the pars distalis of the hypophysis is possible and demonstrable in complete absence of nervous connections. That the still hypothetical hypophysiotrope substance (or substances) of hypothalamic origin, is not epinephrine, norepinephrine, histamine, acetylcholine, 5-hydroxytryptamine, oxytocin, or vasopressin is



Figure 3 Adult acidophils and chromophobes with numerous intra-cytoplasmic granules in outgrowth of a thirty-five-day old tissue culture of anterior pituitary (dog tissue, Azan oil immersion).



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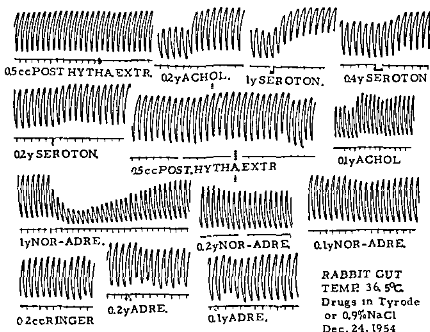


Figure 4 Bioassays for epinephrine, norepinephrine, acetylcholine, 5-hydroxytryptamine (serotonine) of a hypothalamic extract showing ACTH hypophysiotropic activity These graphs were obtained with the physiograph recording system (H E Hoff, L Geddes, and W. Spencer, to be published)

TABLE 3

ACTH-Hypophysiotrope Activity of Saline Homogenates of Various Nuclei of the Hypothalamus (Beef)

Age of Culture	*2 Days*	*2 Days*	*4 Days*	*6 Days*	*8 Days*
Ant pituitary alone	−53±19	+1± 6	+9±27	+39±12	+39±21
Ant pituitary+ Ant hypothalamus	−130±16*	−51±12*		−25±13*	+27±19
Ant pituitary+ Post hypothalamus	−102±15*		−79±21*	−23±11*	−16±19*

Data of several experiments ACTH activity expressed as mean variation of adrenal ascorbic acid concentration in Sayers' test Six to ten hypophysectomized rats per assay

* Indicates results statistically different from control

TABLE 2

ACTH Activity In Fluids of Combined Cultures of the Pituitary and Various Tissues

Fluids from Combined Cultures of:	*Variation in Ascorbic Acid Concentration γ/100 mg Adrenal*	*Degree of Statistical Significance*
Ant pituitary + Post hypothalamus*	−94 ± 11	$p < 0.01$
Ant pituitary + Median eminence*	−188 ± 22	$p < 0.01$
Ant pituitary + Ant hypothalamus*	+41 ± 32	
Ant pituitary + Brain cortex*	+28 ± 15	
Ant pituitary + Spleen*	−31 ± 25	$p \geq 0.05$
Ant pituitary + Liver*	+55 ± 16	
Post hypothalamus alone (12 days)†	+18 ± 7	
Post hypothalamus alone (4 days)*	− 3 ± 10	$p \geq 0.8$

* Dog tissues

† Rat tissues

shown by the negative results of the various bioassays for these substances which were run in fluids of hypothalamic cultures or hypothalamic saline homogenates (*see* Figure 4). The levels of sensitivity of these various assays in our hands are: ≦ 0.1γ to 0.2γ for epinephrine, ≦ 0.05γ to 0.1γ for norepinephrine; ≦ 0.05γ for histamine; ≦ 0.1γ to 0.2γ for 5-hydroxytryptamine, = 1 mU for vasopressin, ≦ 0.5 mU for oxytocin. These results are in agreement with conclusions we reported elsewhere on the basis of pharmacological studies that led us to eliminate epinephrine, norepinephrine, histamine, acetylcholine (Guillemin, 1955) and 5-hydroxytryptamine (Guillemin, unpublished) as possible mediators for the hypothalamic mediation of ACTH release in stress

As with the tissue cultures experiments, the *organ cultures* technique showed us (Table 3) that the whole anterior pituitary *in vitro* does not release ACTH after more than two to four days

age" of the active hypophysiotrope substance (s) from the nuclei where they are actually manufactured to adjacent areas

The definite hypophysiotrope activity in our *in vitro* system of commercial Pitressin, an extract from the posterior lobe of the pituitary, is similarly a challenging finding The posterior pituitary is in direct anatomical relationship with the anterior (supraopticus, paraventricularis) nuclei of the hypothalamus by the supraoptico-hypophysial tract. Enough evidence has been presented for a true secretory activity of these hypothalamic formations (Scharrer, 1954, Hild and Zetler, 1951) so as to imply that the active principles found in the posterior lobe of the pituitary are of hypothalamic origin. Since purified vasopressin shows no ACTH hypophysiotropic activity, this effect of Pitressin should be attributed to a contaminant of vasopressin, likely of hypothalamic origin.

We do not know at the present time if the hypophysiotropic activity found in extracts of both anterior and posterior hypothalamus is due to the same principles There has been recently a series of reports, based on both anatomical (Rothballer, 1953, Scharrer, 1954) and physiological studies (Mirsky, *et al*, 1953, McCann, *et al.*, 1954) holding the posterior pituitary or the anterior hypothalamus responsible for the release of ACTH in stress or at any rate connecting their activity (ADH) with that of the pars distalis (ACTH) It is undoubted from the data presented that there is a constant and remarkable parallelism in the variations of activity of both endocrine systems. However, these experiments fail to demonstrate the causative relationships which would be proof of the validity of their present interpretation. The lack of ACTH hypophysiotropic activity of purified vasopressin in our *in vitro* system is not in favor of ADH as the exclusive and specific mediator of ACTH release

In conclusion, we may say that we have established with simple, easily reproducible methods of tissue or organ culture that the pituitary *in vitro* is unable by itself to release ACTH for any significant length of time, that this ACTH secretion is re-initiated or is considerably increased in amount and duration by combined culture with the hypothalamus or addition of hypothalamic extracts, thus bringing a direct and immediate proof of the possi-

TABLE 4

ACTH-Hypophysiotrope Activity of Commercial Pitressin and Purified Arginine-Vasopressin

Age of Cultures	*2 Days*	*4 Days*
Ant. pituitary, control	-46 ± 16	$+60 \pm 14$
Ant. pituitary + Vasopressin	-60 ± 21	-10 ± 37
Ant. pituitary + Pitressin	-51 ± 12	-59 ± 27*

* Indicates results statistically different from control

ACTH activity expressed as mean variation of adrenal ascorbic acid (γ/100 mg) concentration in Sayers' test Eight animals per assay

of culture, but its secretion of ACTH can be considerably increased and maintained up to eight days of *in vitro* life by incubation with homogenates of the posterior or anterior hypothalamus of bovine origin (Table 3). Homogenates of the brain cortex (ox) have no effect. Commercial Pitressin, even at the small dose utilized in these experiments (less than twice the total amount of vasopressor activity in one rat posterior pituitary), had definite ACTH-hypophysiotrope activity Interestingly enough, purified vasopressin did not increase release of ACTH by the *in vitro* pituitary At the dose utilized here, Pitressin showed no ACTH contamination, by adrenal ascorbic acid depletion test or by injection for ten days to hypophysectomized rats (adrenal weight maintenance test).

The ACTH-hypophysiotropic activity of the homogenates of the anterior hypothalamus, confirmed in several experiments, is in contradistinction with our data in the tissue cultures experiments where only posterior hypothalamus and median eminence tissues showed ACTH-releasing activity. These data are not in agreement with the opinion of the British School headed by Harris on the basis of his results with stereotaxic stimulations and lesions of the posterior hypothalamic region Shall we conclude that the two regions of the hypothalamus may be concerned with ACTH release in different situations which would activate different mechanisms? It might be wise to bear in mind that during the slaughtering process and the time that elapses between stunning and removal of the hypothalamic tissue there might occur a "seep-

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Utilization of *in vitro* pituitary for physiological purposes is still in a very preliminary stage, but it is our belief that as long as we will realize their limitations, these methods will offer the neuroendocrinologist ideal tools to study specific problems in ideally created conditions. The *in vitro* pituitary may be the simple bioassay which will permit a bold approach to the endocrinology of the brain.

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Utilization of *in vitro* pituitary for physiological purposes is still in a very preliminary stage, but it is our belief that as long as we will realize their limitations, these methods will offer the neuroendocrinologist ideal tools to study specific problems in ideally created conditions. The *in vitro* pituitary may be the simple bioassay which will permit a bold approach to the endocrinology of the brain.

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Likewise, the reflex secretion of epinephrine does not appear to be essential to the discharge of ACTH into the circulation.[7]

The hypothesis which is most consistent with known facts is that the stimulation of the anterior pituitary gland and the release of its trophic hormones into the circulation is dependent upon the activation of the hypothalamus [8, 9, 10] Further, it is fairly well established that the activation of the anterior pituitary cannot be due to the transmission of neural impulses from the hypothalamus to the hypophysis and therefore must be due to the transmission of a chemical agent (or agents).[11] Accordingly, it has been proposed by Harris and others [9, 12, 13] that the hypothalamus may secrete a neurohormone (or neurohormones) which is transported from the hypothalamus to the anterior pituitary gland by the hypophysial portal veins which carry blood directly from the hypothalamus to the anterior pituitary gland It is possible also that in response to noxious stimuli, the neurohormone is released into both the hypophysial portal vessels and the general circulation [14, 15]

The chemical nature of the hypothalamic neurohormone is unknown Although acetylcholine,[8] norepinephrine,[16] histamine,[17] and a number of other agents [18, 19] have been suggested, all available evidence indicates that none of these agents is the specific hypothalamic neurohormone. On the other hand, there is a good deal of histochemical evidence that the paraventricular, supraoptic and other nuclei of the hypothalamus are made up of cells which secrete an agent (or agents) This agent has been demonstrated to migrate along the axons of the secretory cells to the median eminence and the neurohypophysis.[20, 21] The neurosecretory material of the hypothalamus has been shown to be associated with the same antidiuretic, pressor and oxytocic properties as are exhibited by extracts of the neurohypophysis All available data indicate that the material secreted by the hypothalamic nuclei is identical pharmacologically with that which can be extracted from the neurohypophysis

Two principal hypotheses have been proposed regarding the site of origin of the neurohypophysial hormones, the antidiuretic hormone (vasopressin) and the oxytocic hormone The commonly accepted hypothesis postulates that vasopressin and oxytocin are

THE RELATION BETWEEN ANTERIOR AND POSTERIOR HYPOPHYSIS AND THE HYPOTHALAMUS IN RESPONSE TO STRESS

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EXPOSURE OF ANIMAL or man to a variety of environmental stimuli results in a sequence of physiological events which are attributable in large measure to the action of the adrenocorticotropic and other hormones of the anterior pituitary gland.[1] The mechanism responsible for the increase of secretion of these hormones is not established. Selye first proposed that some catabolic factor is released from the site of damage and acts directly on the anterior pituitary. Later, he suggested that the gland responds to a variety of metabolic products in the circulation.[2] Sayers proposed that exposure to a noxious stimulus results in an increased utilization of corticosteroids by the damaged tissues, the resultant reduction in the corticosteroid concentration in the blood serving as a 'feed-back' to activate the release of ACTH from the adenohypophysis.[3] Another hypothesis, proposed by Vogt [4] and by Long,[5] is that any situation which results in an increase in ACTH production does so through activation of the sympathico-adrenal medullary system and the direct stimulation of the anterior pituitary gland by the circulating epinephrine.

Although the concentration of corticosteroids may play a role in the maintenance of ACTH secretion, it does not appear to be responsible for initiating the secretion by the anterior pituitary.[6]

hypertonic solution of sodium chloride produces an antidiuretic response,[33] a decrease in the antidiuretic activity of hypothalamic and neurohypophysial extracts[32] and a depletion of the "neurosecretory granules" of the neurohypophysis.[29] The administration of water to dehydrated rats results in restitution of the "neurosecretory granules"[26, 29] and in the antidiuretic activity of extracts of the hypothalamus and neurohypophysis.[26]

In support of the possibility that the 'posterior-pituitary' like hormones of the hypothalamus are involved in the physiological response to noxious stimuli are the classical studies of Verney[34]

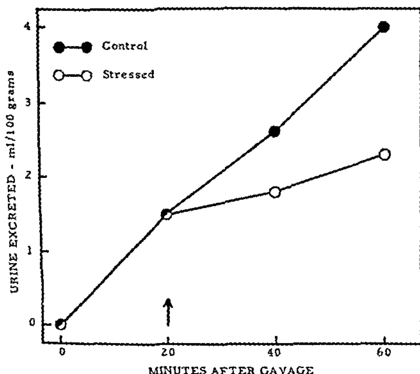


Figure 1 The antidiuretic response of rats to painful stimuli applied to the feet Five ml of 0 2 per cent NaCl per 100 gm. body weight were given at one hour before and again at 0 time At the time designated (↑), one group of twenty-five rats (o—o) was exposed for two minutes to repetitive mild electric shocks to the feet while the other group of forty-two rats was not disturbed (•—•) (From Mirsky, I A, M Stein and G Paulisch *Endocrinology*, 54 491, 1954)

secreted in the neurohypophysis and that such secretion is dependent upon the integrity of the nerve supply from the supraoptic and paraventricular nuclei.[22, 23] The alternative hypothesis attributes the secretion of the hormones of the neurohypophysis to the cells of the supraoptic and paraventricular nuclei while the neurohypophysis acting as the site of storage rather than that of secretion.[20, 21] Smith's observations[24] suggest that the mammillo-infundibular nuclei are also involved in the secretory process. Bodian[25] has extended this concept and proposes that the entire supraoptico-hypophysial system participates in the secretion of the neurohypophysial hormones.

In support of the hypothesis that the supraoptic and paraventricular nuclei are the site of secretion of the neurohypophysial hormones is the demonstration that whereas extracts of the rest of the nervous system are devoid of any antidiuretic activity, extracts of the hypothalamus of various species contain significant amounts of this hormone.[23, 26] The supraoptic nuclei are particularly rich in ADH, containing fifteen to twenty-five per cent as much as does the neurohypophysis.[27] Likewise, extracts with both vasopressin and oxytocic activities have been prepared from the hypothalamic nuclei of many species[26] Even after removal of the neurohypophysis[27] or section of the stalk[26] the hypothalamus contains appreciable quantities of antidiuretic hormone.

Most of the evidence in favor of the hypothalamic origin of the hormones of the neurohypophysis is based on the demonstration of specifically staining "neurosecretory granules" in the supraoptic, paraventricular and mammillo-infundibular nuclei, the supraoptico-hypophysial tract and the neurohypophysis. Bargmann and Scharrer,[28] Leveque and Scharrer[29] and Hild and Zetler[26] have reviewed some of the evidence which indicates that the "neurosecretory granules" are concerned with the secretion, transport, and discharge of the antidiuretic hormone Water deprivation results in an increase in the antidiuretic activity of the urine[30] and plasma,[31] a decrease in the antidiuretic activity of the hypothalamic and neurohypophysial extracts[26, 32] and a disappearance of the "neurosecretory granules" from the neurohypophysis, the supraoptico-hypophysial tract, and the supraoptic and paraventricular nuclei[26, 29] Likewise, the administration of a

each rat in a small metabolism cage to a two-minute period of repetitive mild electric shocks to the feet via a grid which made up the floor of the cage. Such a noxious stimulus produces an inhibition of the diuresis induced by a water load (Figure 1). Within one-half minute after such exposure, the plasma antidiuretic activity rose markedly. Five minutes after such exposure, the ADS titer of the plasma fell somewhat. In ten minutes it was less and in fifteen minutes it was still lower (Figure 2).

Similar studies were performed with rats exposed for five minutes to the noise produced by a Federal siren. Fortier has demonstrated that activation of the adrenal cortex occurs in rats exposed to such noise for thirty minutes. As with pain, exposure to noise for even five minutes resulted in a marked increase in the antidiuretic activity of the plasma (Figure 3). Subsequently, a gradual decrease ensued.

The intraperitoneal injection of 1 mg. of histamine hydrochloride per 100 grams of body weight results in activation of the

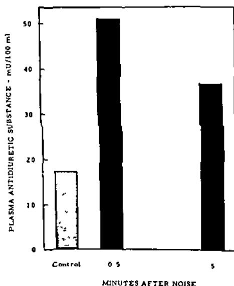


Figure 3 The antidiuretic activity of the plasma of rats exposed for five minutes to the noise produced by a siren (From Mirsky, I. A., M. Stein, and G Paulisch. *Endocrinology*, 54 491. 1954)

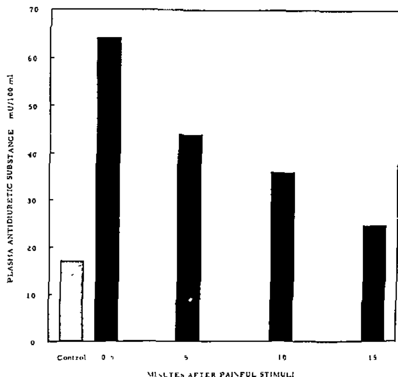


Figure 2 The antidiuretic activity of the plasma of rats exposed *for two minutes to painful stimuli applied to the feet. (From* Mirsky, I A, M Stein, and G Paulisch *Endocrinology, 54* 491, 1954)

which revealed that exposure to physical or emotional noxious stimuli results in an inhibition of the diuresis induced by the ingestion of water A marked diminution in the rate of urine production occurs in animals exposed to such varied stimuli as severe exercise,[35] surgical trauma,[36] mechanical shaking,[37] flashing lights,[38] histamine,[39] and so forth Direct evidence that an increase in the antidiuretic activity of the blood occurs when an animal is exposed to a noxious stimulus became available with the development of a relatively simple, sensitive and precise procedure for the assay of antidiuretic substances (ADS) in the blood plasma [40]

Pain, noise, *injection of histamine, and exposure to a strange* environment were employed as noxious stimuli for evoking an *antidiuretic* response in rats.[41] Pain was produced by exposing

the box. No effect of exposure to the strange environment was noted in rats exposed for less than two minutes The antidiuretic activity of the plasma of rats exposed for two and three minutes, however, was significantly greater than that of rats exposed to the box for shorter intervals (Figure 5). Even after ten minutes of exposure, the activity still remained elevated.

It is pertinent to note that in every known respect, the antidiuretic substance in the plasma behaves like the antidiuretic hormone.[31] Therefore, it appears that the antidiuretic substance which is released into the circulation is identical with the antidiuretic hormone (vasopressin) which can be extracted from the neurohypophysis.

Having demonstrated that sensory stimuli (pain and noise), systemic stimuli (histamine), and emotional stimuli (strange environment) induce a rapid secretion of an antidiuretic substance which resembles the antidiuretic hormone, it became pertinent

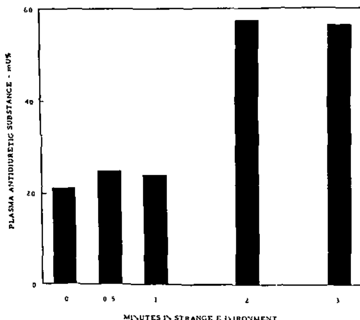


Figure 5 The antidiuretic activity of the plasma of rats exposed to a strange environment (From Mirsky, I A, M Stein, and G Paulisch *Endocrinology, 54* 491, 1954)

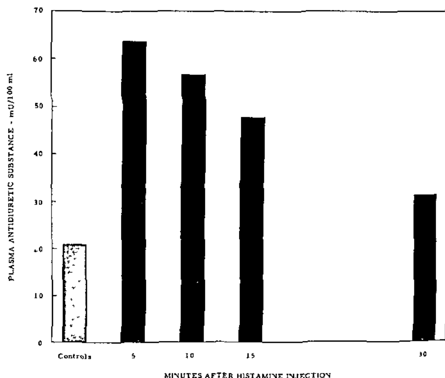


Figure 4 The antidiuretic activity of the plasma of rats given an intraperitoneal injection of histamine (From Mirsky, I A, M Stein, and G Paulisch *Endocrinology, 54* 491, 1954)

anterior pituitary gland of rats The same quantity of histamine produced a marked increase in the antidiuretic activity of the plasma within five minutes after the injection (Figure 4). Thereafter, the titer fell, so that by thirty minutes the antidiuretic activity of the plasma was similar to that of the saline-injected animals

In order to test the effect of an emotional stimulus on the antidiuretic response, rats were placed individually for variable periods of time into a two compartment *conditioning* box which may be regarded as a strange environment for rats Introduction of rats into the box produced no overt behavioral responses other than defecation and an increase in grooming activities such as have been utilized by some investigators as a measure of emotionality of rats As controls, rats were handled but not put into

We then studied the effect of hypophysectomy and demonstrated that in from five to ten days after removal of the whole pituitary, *there is a statistically significant diminution in the antidiuretic activity of the plasma.* Similar hypophysectomized rats were then exposed to noxious stimuli Like normal animals, the completely hypophysectomized rats responded to noise and to painful stimuli with a rapid, marked discharge of ADS into the circulation (Figure 8). Consequently, neither the anterior nor posterior pituitary is essential to the release of the ADS into the circulation.

In order to establish that the observations on rats are not specific for these animals, studies were performed on man.[42] For *example, healthy men were given 1000 ml. of tap water and the* urine that was voided every ten minutes was replaced with an

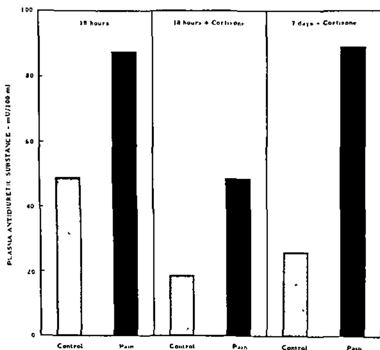


Figure 7 The antidiuretic activity of the plasma of cortisone-treated and untreated adrenalectomized rats exposed to painful stimuli (From Mirsky, I A, M Stein, and G. Paulisch *Endocrinology*, 55 28, 1954)

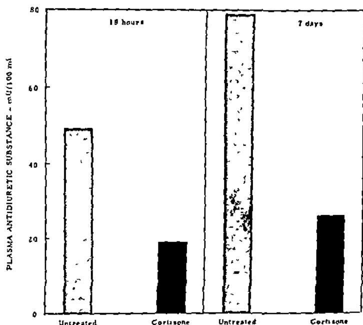


Figure 6 Effect of treatment with cortisone on the antidiuretic activity of the plasma of adrenalectomized rats (From Mirsky, I A, G Paulisch, and M Stein *Endocrinology*, 54 691, 1954)

to demonstrate the roles of the adrenal and pituitary glands in this process [40, 41] Towards this end rats were adrenalectomized and at eighteen hours and seven days after the operation, the antidiuretic activity of the plasma was determined and found to be significantly increased The administration of cortisone prevented the increase in the antidiuretic activity of the plasma of such animals (Figure 6)

The response of adrenalectomized rats to painful stimuli was then determined. Although the activity of the plasma of the untreated eighteen-hour adrenalectomized rats was significantly greater than that of normal rats, exposure to painful stimuli resulted in a further increase Such exposure also increased significantly the antidiuretic activity of the plasma of eighteen-hour and seven-day cortisone treated adrenalectomized animals (Figure 7) Consequently, the adrenal gland is not essential to the antidiuretic response to noxious stimuli

observation that a surgical operation induces a marked augmentation in the antidiuretic activity of the plasma.[43] A maximum titer was observed immediately after the operation, thereafter there was a gradual reduction so that by two to three hours after the operation the plasma ADS titer was back to the pre-operative level. In contrast is the effect of such an operation on the rate of release of adrenal cortical hormones in that the maximum titer of adrenocorticosteroids is attained only by the time the ADS has returned to a minimal concentration.[43]

Like with surgical procedures, it has been demonstrated that *electric shock, insulin hypoglycemia,* and a *variety of other noxious stimuli* can result in the release of ADS into the circulation of man which only much later is followed by an increase in adrenal cortical activity.[43]

The same noxious stimuli which elicit an antidiuretic re-

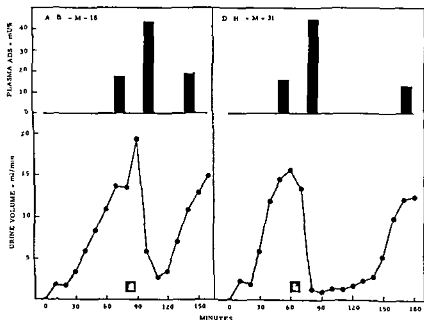


Figure 9 The effect of a noxious stimulus on diuresis and on the antidiuretic activity of blood plasma Ischemic pain of an arm was produced during the period marked by the shaded area (From Mirsky, I A, and M Stein *Science, 118* 602, 1953)

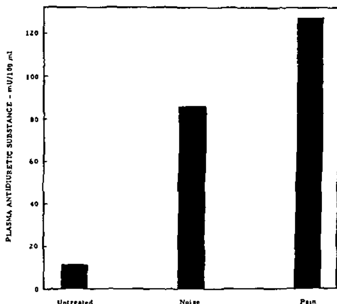


Figure 8 The antidiuretic activity of the plasma of

equal volume of water in order to maintain a constant water load. When the rate of urine excretion exceeded a volume of 10 ml. per minute for two consecutive periods, the circulation through the right arm was occluded by a sphygmomanometer cuff and ischemic pain induced At the end of nine minutes, the cuff was deflated and the subject permitted to void by the tenth minute. The production of ischemic pain of the arm resulted in a marked inhibition of diuresis within ten minutes after the cessation of pain The duration of the antidiuresis varied from individual to individual and seemed to be related to the degree of anxiety exhibited by the subject Minimal quantities of ADS were found in the plasma prior to the production of pain. A significant increase in the antidiuretic activity of the plasma was noted by the time maximal inhibition of water diuresis occurred (Figure 9). The sample drawn after the restitution of the diuresis showed a return of the plasma ADS to its pre-test concentration.

The effect of noxious stimuli in man is revealed also in the

observation that a surgical operation induces a marked augmentation in the antidiuretic activity of the plasma.[43] A maximum titer was observed immediately after the operation, thereafter there was a gradual reduction so that by two to three hours after the operation the plasma ADS titer was back to the pre-operative level. In contrast is the effect of such an operation on the rate of release of adrenal cortical hormones in that the maximum titer of adrenocorticosteroids is attained only by the time the ADS has returned to a minimal concentration.[43]

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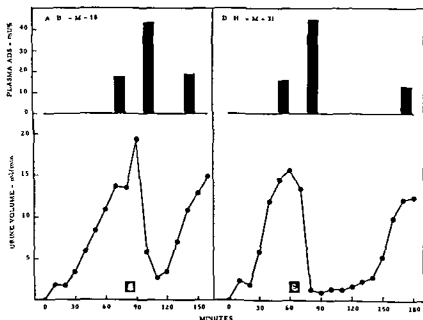


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sponse and an increase in the antidiuretic activity of the plasma in normal, adrenalectomized, and hypophysectomized rats can initiate also the sequence of events described by Selye[1] as the "alarm reaction." A decrease in the rate of urine excretion and in the diuretic response to the ingestion of a water load is noted only during the early "shock" phase of the "alarm reaction." Thereafter, with the appearance of other manifestations of an increased adrenal cortical activity, the output of urine may become normal or even excessive. In the absence of the adrenals, the stimuli which initiate the "alarm reaction" may result in a more intense oliguria than when the adrenals are intact.[37, 38, 44] The oliguria of the "alarm reaction," therefore, is independent of and precedes the activation of the adrenal cortex. Since an increase in the reabsorption of water by the renal tubules is a major factor in the oliguria of the rat with adrenal insufficiency,[45] it may be assumed that the oliguria is due in part at least to the release of ADH into the circulation. In accord are the observations that exposure to noxious stimuli result not only in an inhibition of diuresis induced by a water load but also in a decrease in the antidiuretic activity of hypothalamic and neurohypophysial extracts[32] and an increase in the antidiuretic activity of the plasma of the rat.[41] The rapidity with which the "neurosecretory" material of the hypothalamus is mobilized and enters both the general circulation and the hypophysial portal vessels,[15] the rapidity with which the antidiuretic activity of the plasma is increased in the normal or adrenalectomized rat[31, 41] and the relatively long interval between the appearance of ADS and 17-hydroxycorticosteroids in the circulation of man[43] favor the hypothesis that the secretion of ADH precedes the activation of the adrenal cortex

Since the discharge of ADH appears to precede the activation of the adrenal cortex and since the release of adrenocorticotrophic hormone (ACTH) is essential to the latter, it is possible that the discharge of ADH by the hypothalamus and the discharge of ACTH by the adenohypophysis are related Both events occur in response to noxious stimuli or to adrenalectomy. Both events occur at a very rapid rate: the ACTH content of the blood "is significantly increased within two minutes after subjecting animals to stressful stimuli,"[46] while the antidiuretic ac-

tivity of the plasma is likewise maximal by two minutes after the onset of such stimuli.[41] Finally, a relationship between the discharge of ADH into the circulation and the activation of the adenohypophysis is suggested by the decrease in adrenal ascorbic acid which ensues after the administration of vasopressin to rats.[42, 45, 49] *The administration of equivalent amounts of oxytocin, however, does not produce a depletion of adrenal ascorbic acid.*[50]

The more recent studies by McCann and Brobeck[49] and by McCann and Sydnor[51] extend the aforementioned observations and provide more definitive evidence that the secretion of ACTH is related to the secretion of vasopressin by the hypothalamus. These investigators demonstrated that lesions of the hypothalamus which involve a significant portion of the supraoptico-hypophysial tract and result in diabetes insipidus also block the secretion of ACTH as measured by adrenal ascorbic acid depletion, adrenal weight, or blood ACTH concentration.

Stimuli which result in a discharge of ADH into the circulation may induce a concomitant discharge of oxytocin,[52, 53, 54] and a reduction in the oxytocic activity of hypothalamic and neurohypophysial extracts.[29] The quantity of hormones discharged may depend upon the type of stimulus. Thus, Cross[52] noted a slight antidiuretic and a marked oxytocic response (milk-ejection) in lactating rabbits suddenly exposed to their suckling young. Andersson[53] demonstrated that the injection of a hypertonic sodium chloride solution which results in a marked antidiuretic response[31] produces also a discharge of oxytocin. If the antidiuretic and vasopressin activities are due to a single agent, then there are at least two distinct polypeptides, vasopressin and oxytocin[55] which may be secreted by the hypothalamus and discharged from the hypothalamic-neurohypophysial system *into the general circulation and into the hypophysial portal vessels after exposure to noxious stimuli. It is quite possible that one of these agents acts specifically on the acidophilic and the other on the basophilic cells of the adenohypophysis.*

The aforementioned considerations support the hypothesis that exposure to various environmental stimuli result in activation of the hypothalamus and the secretion of vasopressin and oxytocin

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by the hypothalamic nuclei. These hypothalamic agents then serve as the 'neurohormones' responsible for the activation of the adenohypophysis which ensues in response to noxious stimuli.

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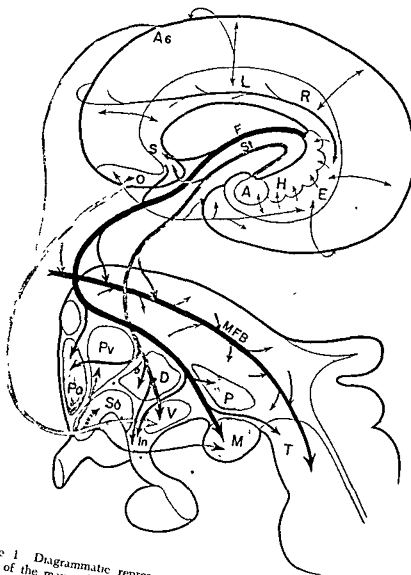


Figure 1 Diagrammatic representation of the main telencephalo-hypothalamic connections

A amygdala A6 Area 6 of frontal lobe
D dorsomedial nucleus of hypothalamus
E entorhinal area (piriform lobe)
F fornix
In infundibulum
L limbic (cingulate) gyrus
M mammillary body
MFB medial forebrain bundle
O orbito-frontal cortex
P posterior hypothalamus
Po preoptic region
Pv paraventricular nucleus
R retrosplenial and posterior cingulate region
S septal area (subcallosal region)
So supraoptic nucleus
St stria terminalis
T tegmentum

The arrows indicate the direction of nervous impulses in this system The arrows in the diagram of the hemisphere outline the main associational connections at the cortical level, which are relevant for this system

TELENCEPHALIC INFLUENCES UPON THE HYPOTHALAMUS

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INTRODUCTION

It may seem premature to review the telencephalo-hypothalamic relations in terms of their significance for the integration of neuroendocrine mechanisms, since to my knowledge no extensive studies specifically related to this particular problem have been carried out up to the present time. It may therefore appear that in the present review little will have a very direct bearing on the main subject of this symposium. However, for the guidance of future research in this field, a functional concept of the relations of higher cerebral centers to the hypothalamus as developed on the basis of our present knowledge seems to be a necessary prerequisite. This may be taken as the justification for the present review Its aims will be to give a general survey of the work done on telencephalo-hypothalamic relationships with some emphasis on personal studies and to try to develop some general ideas concerning their significance in the over-all integration of global functional patterns of animal and human behavior.

ANATOMICAL BASIS (Figure 1)

The best way to understand the basic pattern of corticosubcortical interrelationships is to study their phylogenetic evolution. The same is true for the more specific question of telencephalo-hypothalamic connections The hypothalamus is a phylogenetically old brain structure and it appears therefore

(3) The mesocortex, comprising the orbito-frontal, anterior insular and the cingular cortex.

(4) The archistriatum or amygdala

(5) The paleostriatum or globus pallidus

These structures receive neo-cortical (MacLean and Pribram, 1953; Pribram and MacLean, 1953) and neo-striatal afferent connections (Wilson, 1914; Ranson and Ranson, 1942; Mettler, 1945) respectively, which suggests that they may act as an intermediary between the newer part of the endbrain and the older part of the diencephalon—the hypothalamus and the habenular system (epithalamus)—to which these new parts, with the already mentioned exception of area 6, have otherwise no direct access.

The individual components of this old telencephalic system are closely interconnected at the cortical level mainly through two well known cortical associational tracts, the cingulum and the uncinate fasciculus

According to Pribram and MacLean (1953) this whole cortical system around the hilus of the hemisphere can be subdivided into five main sectors on the basis of the mutual interrelationships of their constituent parts and their reciprocal relationship with adjacent neocortical areas.

(1) The fronto-temporal region, comprising the orbito-frontal, anterior insular, temporal polar, and piriform cortex

(2) The medial occipito-temporal region, comprising the hippocampal, fusiform, and lingual gyri

(3) The medial parieto-occipital region, consisting of the precuneus, the retrosplenial, and the posterior limbic cortex.

(4) The medial fronto-parietal region, consisting of the anterior limbic and superior frontal cortex

(5) The medial frontal region consisting of the subcallosal gyri, the medial orbitofrontal and the ventro-medial frontal polar cortex

Moreover, the amygdala is interconnected in a reciprocal way with the overlying piriform cortex (Johnston, 1923, Hilpert, 1928, Crosby and Humphrey, 1944, Lauer, 1945, Pribram and MacLean, 1953, Gloor, 1955). The posterior part of the latter, the entorhinal area, is the most important afferent pathway to

likely that its direct descending afferent fibers will originate in the oldest parts of the cortex and basal ganglia. This is indeed what comparative anatomy shows. The oldest afferences to the hypothalamus from telencephalic formations are the primordial fornix system originating in the primordium hippocampi and the primordial stria terminalis system originating from the primordium of the striatum and amygdala This is the situation as it is seen in cyclostomes (Crosby and Woodburne, 1940) and remains the basic pattern throughout the phylogenetic scale up to man. In mammals some less conspicuous additional cortico-hypothalamic pathways are found They originate in the orbito-frontal cortex (Ward and McCulloch, 1947; LeGros Clark and Meyer, 1950, Beck, Meyer and LeBeau, 1951; Wall, Glees and Fulton, 1951) in area 6 of the frontal lobe (Ward and McCulloch, 1947, Meyer, 1949, LeGros Clark and Meyer, 1950, Beck, Meyer and LeBeau, 1951), and in area 32 or 25 in the most anterior part of the cingular cortex (Nauta, 1955). With the exception of area 6, which is truly neocortical, these areas have to be classified as mesocortex (M Rose, 1927; J E Rose, 1942), a relatively old cortex representing a sort of transitional stage between the oldest types of pallium as represented by archi- and paleo-cortex and the phylogenetically young neocortex. It is quite conceivable that these last named cortico-hypothalamic connections may have forerunners in submammalian forms, but since knowledge of these systems is fairly recent, no studies concerning their comparative anatomy are yet available.

On the basis of comparative anatomical studies, it becomes therefore evident that it is mainly the ring of old cortex surrounding the hilus of the hemisphere and the old striatum, which represent the telencephalic formations closely connected with the hypothalamus

These old telencephalic formations comprise the following structures

(1) The archicortex or hippocampus

(2) The paleocortex or cortex of the piriform lobe, grossly corresponding to the cortex of the uncus and of the hippocampal gyrus in man.

of the vertebrates from cyclostomes up to man and which is probably of great physiological importance.

Besides this common characteristic of the main telencephalo-hypothalamic connections there are, however, patterns more specific for each individual system

The fornix relaying hippocampal impulses to the hypothalamus can be followed as a well defined fiber bundle down to the mammillary bodies, where it terminates mainly in the medial mammillary nucleus and much less in the lateral mammillary nucleus (LeGros Clark and Meyer, 1950, Brodal, 1947; Nauta, 1955). A great number of fornix fibers, however, leave the main course of the bundle all the way down its course from the septum onward and end in various septal, thalamic, and hypothalamic nuclei and areas (Gudden, 1880, O. Vogt, 1898, Humphrey, 1936, Young, 1936, Fox, 1940, Allen, 1944, Brodal, 1947, Nauta, 1955). Terminals have been found by Nauta (1955) in the anterior, midline, and intralaminar thalamic nuclei in the lateral preoptic area and in the lateral hypothalamic area down to the level of the premammillary region. These fibers to the lateral hypothalamus and some which can be traced into the mesencephalic tegmentum are interrupted by at least one synaptic station in the septum Moreover, fornix terminals are found in the dorsal hypothalamic area, some in the posterior hypothalamus and especially in the periventricular zone of the anterior hypothalamus sparing the paraventricular nucleus and from here down to the medial part of the arcuate or tubero-infundibular nucleus No connections are found to the ventro-medial, nor to the dorsomedial hypothalamic nuclei (Nauta, 1955)

The amygdala projects directly to the preoptic area, the anterior hypothalamus, the dorsomedial, ventromedial, and arcuate nuclei of the infundibular portion of the hypothalamus (Johnston, 1923, Smith, 1930, Young, 1936, Humphrey, 1936, Fox, 1940, 1943, Adey and Meyer, 1952, Nauta, 1955, Gloor, 1955) Electrophysiological studies, however, have shown that the amygdala is capable of activating through multi-synaptic relays a more extensive area extending from the septum through the hypothalamus and subthalamus back to the rostral mesencephalic tegmentum (Gloor, 1955) This may represent the activity me-

the hippocampus (Cajal, 1911; Lorente de No, 1933, 1934, Allen, 1948).

These old cortical and striatal formations funnel down their activity to the hypothalamus through the following main systems:

(1) The hippocampo-hypothalamic system discharging over the fornix.

(2) The amygdalo-hypothalamic system running through the stria terminalis and other shorter amygdalo-hypothalamic connections.

(3) The orbito-hypothalamic system

(4) The pallido-hypothalamic tract (Bard and Rioch, 1937). (This system will not be taken in account in the further course of this review since little, if anything, is known about its function.)

Part of the fibers originating in the hippocampus and the amygdala are interrupted in their downward course at the level of the septal nuclei acting as relay stations in these cortico- and amygdalo-hypothalamic pathways In man the subcallosal gray matter is said to represent the homologue to the septal nuclei of subhuman species (Kappers, Huber and Crosby, 1936, Brodal, 1947; Heath, 1954)

At the hypothalamic level these systems show some similarities, but also some specific differences in projection patterns. The similarities consist in the fact that all of them enter in relation with the medial forebrain bundle (Crosby and Woodburne, 1940, Fox, 1940, LeGros Clark and Meyer, 1950, Young, 1936, Brodal, 1947, Nauta, 1955, Gloor, 1955), which is the main longitudinal associational system of the hypothalamus reaching as far caudal as the mesencephalic tegmentum This system contains long and short (Auer, 1953) neurons making up a rather complex and diffuse associational and projection system distributing fibers to and receiving connections from almost all hypothalamic regions and nuclei This close association of the telencephalo-hypothalamic projection apparatus with the system of the median forebrain bundle is a situation which remains basically unchanged throughout the whole phylogenetic scale

will call "limbic system" from now on. Excellent reviews of this subject have been written by Kaada (1951), Fulton (1951), Gastaut (1952), and Pribram and Kruger (1954), together with the presentation of their own experimental material Only a brief summary of their experimental results, together with some reported by other investigators, will be given here.

Two general statements characterizing the functional patterns elicited by limbic stimulations can be made.

(1) The whole gamut of responses of hypothalamic stimulation can be reproduced in a somewhat sketchy form from this area

(2) The pattern of responses obtained from any individual part of this archaic telencephalic system is very similar to that obtained from other parts of the same system Some exceptions to this rule are present, and will be dealt with presently, but in comparison to the similarity of functional patterns otherwise encountered as a rule throughout the whole limbic system, they appear as of secondary importance and do not invalidate the statement that the functional characteristics of all constituent parts of the limbic system seem to be essentially similar

The exceptions to these rules, which were just mentioned, concern first the hippocampus and the retrosplenial portion of the limbic cortex According to Kaada, Jansen and Andersen (1953) these areas do not yield autonomic nor elementary somato-motor responses, but produce only the "attentive" behavioral responses, which, however, are not specific for these regions since they can also be obtained from all other subdivisions of the limbic system It should, however, be emphasized at this point that the findings of Kaada and co-workers (1953) though duplicated to some extent by results reported by MacLean and Delgado (1953) could not be confirmed by Akert and Andy (1953) in their careful studies on animals nor are they supported by observations in humans (Penfield and Jasper, 1953) Akert and Andy (1953) found the hippocampus conspicuously silent on electrical stimulation, to the point even that normal reactivity of the animal to environmental stimuli was fully retained even at the height of a far-spreading hippocampal seizure discharge.

diated by the system of the medial forebrain bundle. It is conceivable that the other telencephalo-hypothalamic systems may act in a similar way as the amygdala since they are also related to the medial forebrain bundle

The orbito-frontal cortex and also the frontal cortex of area 6 are mainly connected to the ventromedial hypothalamic nucleus (*LeGros Clark and Meyer, 1950; Beck, Meyer and LeBeau, 1951*; Wall, Glees and Fulton, 1951). Connections from these frontal areas to the supraoptic and paraventricular nuclei have also been demonstrated (Ward and McCulloch, 1947; Wall, Glees and Fulton, 1951). Area 6 also seems to project to the medial mammillary nucleus (Ward and McCulloch, 1947; Meyer, 1949, LeGros Clark and Meyer, 1950).

Thus the hypothalamus receives a rather complex pattern of afferent connections from higher brain areas, but in turn there are also hypothalamo-telencephalic systems providing the basis for reverberating circuits and feedback mechanisms.

They will only be mentioned rather briefly here.

(1) The mammillo-thalamo-cortical pathway connecting the medial mammillary nucleus with the limbic cortex with a relay station in the anterior thalamic nuclei This pathway is a phylogenetically *recent acquisition and appears only in mammals* where it develops in parallel to the differentiation of a specialized hippocampo-mammillary pathway as represented by the columns of the fornix (LeGros Clark and Meyer, 1950, Brodal, 1947)

(2) The hypothalamo-frontal pathway connecting the hypothalamus to the frontal cortex through a periventricular fiber system via the dorsomedial nucleus of the thalamus (Roussy and Mosinger, 1933, Walker, 1936, Murphy and Gellhorn, 1945)

(3) A diffuse projection system to the cortex originating in the posterior hypothalamus and the adjacent mesencephalic reticular formation as described by Moruzzi and Magoun (1949) This system exerts important functions in the maintenance of consciousness

STIMULATION STUDIES

It is not within the scope of this review to give a detailed account of all the results obtained by electrical stimulation within this old telencephalic system which for the sake of simplicity we

anisms the respiratory changes, which though not mediated through autonomic fibers in the periphery, are from a functional point of view vegetative in nature.

Respiration is mainly inhibited from almost the whole extent of the limbic system Akert, Hess and McDonald (1951) report, however, that respiratory activation is the rule on stimulation of the cingulate and orbital gyri, when the unanesthetized animal is stimulated through implanted electrodes. Inhibition on the contrary is the rule under anesthesia. In cats and dogs and also occasionally in monkeys, respiratory acceleration is obtained from the posterior part of the anterior cingulate area (Spencer, 1894, Tower, 1936, Smith, 1938, Bailey and Sweet, 1940, Hodes and Magoun, 1942, Smith, 1945, Ward, 1948; Livingston, *et al*, 1948; Delgado and Livingston, 1948, Sugar, Chusid and French, 1948; Kaada, Pribram and Epstein, 1949; Sachs, Brendler and Fulton, 1949, Speakman and Babkin, 1949, Pool and Ransohoff, 1949, Kaada, 1951; Hess, Akert and McDonald, 1951; Gastaut, 1952; Koikegami and Fuse, 1952, MacLean and Delgado, 1953, Kaada, Andersen and Jansen, 1954).

Cardiovascular responses produced by limbic stimulation can be either sympathetic or parasympathetic or mixed Changes in the rate of heart beat are not often reported, but blood pressure changes are very common There is no topographical distinction between areas producing a raise in blood pressure and others eliciting a drop in arterial pressure. Pressor and depressor points are intermingled (Figure 2). The direction of the blood pressor response seems to depend to some extent at least upon the general condition of the animal, as e g., on the level of anesthesia Depressor effects are more likely to occur under deep anesthesia, pressor responses are easier to elicit under light anesthesia. Reversals of the blood pressure responses have also been seen to occur when the frequency of stimulation or the form of the stimulating pulse were changed (Spencer, 1894, Smith, 1938, Bailey and Sweet, 1940, Smith, 1945, Delgado and Livingston, 1948, Speakman and Babkin, 1948, Ward, 1948, Sachs, Brendler and Fulton, 1949, Kaada, 1951, Hess, Akert and McDonald, 1951, Gastaut, 1952, Koikegami, Kimoto and Kito, 1953)

Several authors (Bailey and Sweet, 1940, Babkin and Kite,

Penfield and Jasper (1953) observed that stimulation of the hippocampus in humans fails to produce any observable effects in conscious patients, who in no way are aware of any change induced by hippocampal stimulation. We therefore feel that the functions of the hippocampus are still shrouded in mystery and it may be well justified to make some reservations concerning its close association with the remainder of the limbic system The concept of limbic functions as developed in this review should therefore only be applied to the hippocampus proper in a very tentative way. Similar reservations may be made concerning the functions of the retrosplenial and posterior cingulate cortex.

Another exception concerns "oral" activities like licking and mastication obtained from the orbito-temporal portion of the limbic system including the amygdala. This orbito-temporal portion, however, participates as well in all the other somato-autonomic and behavioral mechanisms as activated from the remainder of the limbic system. Feindel and Penfield (1954) in *their study on temporal lobe automatism point out that in the* conscious patient undergoing surgery for temporal lobe epilepsy stimulation of the temporal part of this orbito-temporal portion *of the limbic system induces a confusional state with amnesia,* which is identical to the changes observed in epileptic automatism. This may suggest that activity originating in this part of the limbic system is particularly apt to interfere with the reticular brain stem mechanisms involved in the maintenance of consciousness.

Some responses from the amygdala and the overlying cortex should also be mentioned here They consist in sniffing and related olfactory-type of behavioral responses (Kaada, Andersen and Jansen, 1954, Gastaut, 1952, 1953, MacLean and Delgado, 1953). They may represent a different order of activities from those which are the object of this study and are probably related to the specific olfactory system

A brief review of the responses obtained by limbic stimulation will illustrate these general statements

In the sphere of autonomic responses elicited by limbic stimulation we find a mixture of sympathetic and parasympathetic effects We may also include here among the autonomic mech-

Pupillary changes are very common responses to limbic stimulation. There is almost always dilatation of the pupils from this area of old cortex and from the amygdala. Only stimulation of the limbic cortex just in front of the genu of the corpus callosum is reported to produce pupillary constriction (Hodes and Magoun, 1942; Smith, 1945; Ward, 1948, Sachs, Brendler and Fulton, 1949, Kaada, 1951; Gastaut, 1952; Koikegami and Yoshida, 1953).

Other autonomic responses obtained from this area, which, however, have not been analyzed so thoroughly as the aforementioned, include defecation and micturition (Kaada, 1951, Gastaut, 1952; Kremer, 1947; Henneman, 1948), activation of uterine tonus and contraction (Koikegami, *et al.*, 1954), piloerection (Hoff and Green, 1936, Smith, 1945, Ward, 1948, Kaada, 1951), and salivation (Kaada, 1951).

This wide range of autonomic responses obtained by limbic stimulation makes it impossible to speak at this level any longer in terms of a dualistic organization of the autonomic system. Here the distinction between a sympathetic and parasympathetic system has faded away. The best illustration for this is perhaps the blood pressor responses, which from the same zone can be either pressor or depressor (Figure 2).

The rich variety of autonomic responses obtained from this old telencephalic system has induced MacLean (1949, 1952) to call it a "visceral brain," a term which has been criticized by Pribram (1952) and Gastaut (1952). It is indeed a somewhat misleading designation, since the functional responses obtained from the limbic system are by no means exclusively or even predominantly autonomic but somatic as well. The somato-motor responses obtained by limbic stimulation differ, however, from the specific motor responses as obtained from neocortical structures and rather resemble the motor effects and influences upon the motor system as observed in hypothalamic and brain stem tegmental stimulation

Inhibition of muscle tone and of spontaneous movements together with suppression of motor after-discharges were seen in lightly anesthetized preparations, whereas muscle tone was increased together with arrest of spontaneous motor activity in unanesthetized freely moving animals stimulated through implanted electrodes (Kaada, 1951; Gastaut, 1952).

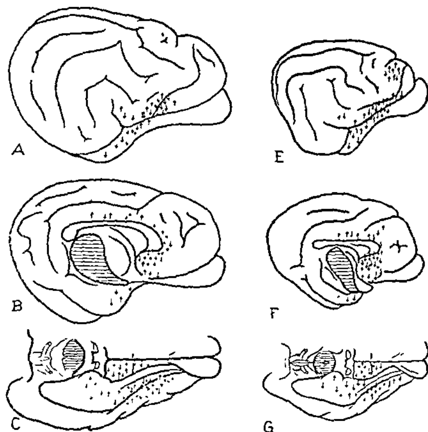


Figure 2 Blood pressure responses obtained from cortical stimulation Arrows indicate direction of blood pressure response Note complete overlap of pressor and depressor points (From Kaada *Acta Physiol scandinav*, 1951)

1950, Babkin and Speakman, 1950, Kaada, 1951, Eliasson, 1952; Koikegami, *et al*, 1953) described gastro-intestinal responses to stimulation of various points within the limbic system Inhibitory and excitatory responses have been described For the mesial temporal region adjacent to the hippocampal gyrus, Kaada (1951) was able to demonstrate that the type of response was conditioned by the pre-stimulatory state of the effector organ, high tonicity and active peristalsis seemed to favor inhibitory responses, low tonicity and weak peristalsis was more likely to favor activation of the smooth musculature of the pyloric-antral portion of the stomach

lation of limbic structures upon endocrine mechanisms. There is only scattered information on this subject, which indicates that the limbic system may be able to activate the release of gonadotrophic hormone and ACTH from the pituitary through hypothalamic mechanisms

Koikegami and co-workers (1954) report that amygdaloid stimulation produces ovulation in the female rabbit. The response is almost constant during estrus but still very frequent in the anestrus phase of the cycle.

Heath and his collaborators (1954) on stimulation of the septum obtained responses suggesting release of ACTH from the pituitary. Their evidence was mainly based on a drop in eosinophils and lymphocytes following septal stimulation.

If these endocrine effects are specific responses of the septum or the amygdala respectively, or if they can be obtained from other parts of the limbic system, as one might surmise, is still an open question.

Electrical stimulation in any part of the limbic system may also produce diffuse electrocorticographic effects, which are best described as an activation pattern characterized by low voltage fast activity (Sloan and Jasper, 1950, Kaada, 1951, Feindel and Gloor, 1954). This pattern is very similar, if not identical, with that produced by stimulation of the brain stem reticular formation and of the posterior hypothalamus, as described by Moruzzi and Magoun (1949), and has been correlated with the behavioral state of arousal or vigilance

In unanesthetized animals stimulation of limbic structures through implanted electrodes induces complex behavioral patterns. The most thorough studies in this field have been carried out by Gastaut (1952), by Kaada, *et al* (1953, 1954), and by MacLean and Delgado (1953) The responses are characterized by an "attentive" behavior, which may give way to anxiety and fear or even rage, especially on raising the intensity of the electrical stimulation To several observers the behavior displayed by these animals suggests that they experience some sort of hallucinations (Kaada, *et al*, 1943, 1954) It certainly resembles the behavioral patterns produced by Hess on stimulation of the perifornical and posterior hypothalamic regions in cats (Hess, 1949, 1954, Hess and Brugger, 1951)

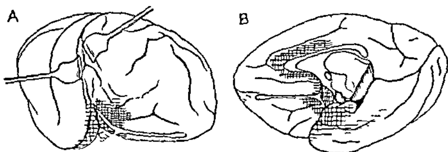


Figure 3 Effect of "limbic" stimulation upon cortically induced movements. Vertical lines indicate facilitation, horizontal lines inhibition of cortically induced movements *Note almost complete topographical overlap of both types of responses* (From Kaada *Acta Physiol. scandinav.*, 1951)

When limbic stimulation effects upon motor activities are tested on cortically induced movements or on spinal monosynaptic and polysynaptic reflexes, three types of responses may be observed facilitation, inhibition, or facilitation changing into inhibition *while stimulation goes on. There is a complete topo*graphical overlapping of these three types of responses (Figure 3), (Kaada, 1951).

Overt motor responses are also obtained from the limbic area They usually involve masticatory and facial movements and are especially apt to occur from the orbito-temporal portion of the limbic system including the amygdala (Kaada, 1951, Gastaut, *1952, MacLean and Delgado, 1951, Hess and Akert, 1951*)

Another motor response occasionally observed is vocalization It is obtained in monkeys from the anterior cingulate and from the anterior hippocampal gyral area (Kaada, 1951, Smith, 1945)

The over-all characteristics of these somato-motor responses give us again a picture similar to that observed for the autonomic effects There is no specificity of action, with the possible exception of masticatory *responses and vocalization, which show a* somewhat more restricted topographical representation Again the character of most of the responses is rather temperamental, as evidenced by the fact that the same area can produce inhibition, facilitation, or inhibition changing into facilitation of evoked motor activities (Figure 3).

Very little is known of the possible effect of electrical stimu-

It also shows on stimulation the whole range of the simpler autonomic, somatic, and electrocorticographic patterns of limbic activity (Kaada, 1951; Gastaut, 1952, Koikegami, *et al.*, 1952, 1953, 1954; Kaada, Andersen and Jansen, 1954; Feindel and Gloor, 1954) and may therefore be regarded as truly representative of the functional properties of the limbic system.

The amygdala is made up of several subnuclei, which may be grouped into two main complexes, the phylogenetically older cortico-medial group and the phylogenetically younger baso-lateral group of nuclei (Johnston, 1923; Holmgren, 1925, Humphrey, 1936). The former discharges mainly over the stria terminalis into its subcortical projection areas and the latter with the possible exception of the posterior part of the basal nucleus, which may feed fibers into the stria terminalis (Fox, 1940, 1943), projects over shorter anatomically ill-defined routes into its subcortical projection field. Anatomically, the projection fields from the cortico-medial and baso-lateral amygdala seem to overlap rather extensively and the final terminations of the fibers arising in the amygdala have been said to reach the septal nuclei, the preoptic and anterior lateral hypothalamic area, the ventromedial, the dorsomedial, and the arcuate nuclei of the hypothalamus Fibers of amygdaloid origin also seem to contribute to the medial forebrain bundle (Johnston, 1923, Smith, 1930, Young, 1936, Humphrey, 1936, Fox, 1940, 1943; Adey and Meyer, 1952; Nauta, 1955).

This pattern of projection was confirmed in our experiments (Gloor, 1955). A grouping of short latency responses to electrical stimulation of the amygdala was found in the just-named regions (Figure 4A). It also confirmed that projection patterns from the cortico-medial and baso-lateral subdivisions of the amygdala are not very much different. Fibers from the former reach probably somewhat farther caudal in the hypothalamus than those from the latter and may go as far back as the posterior hypothalamus. The ventro-medial nucleus is probably only directly connected with the cortico-medial amygdaloid subdivision.

More interesting, however, is the consideration of the long latency responses (Figure 4B), which are recorded from a very wide subcortical area extending from the septum back to the

An attempt to organize the variety of these stimulation-responses into a meaningful context is a difficult undertaking. One is most likely doomed to failure if such an attempt is based on the assumption that functional representation in the limbic system is based on the same principle of topographical representation of separate specific functions in separate zones, as it is the case in the neocortex. All experimental evidence so far seems to contradict the presence of such a type of organization and the anatomy of the projection pathways originating in the limbic system does not show any evidence that projection patterns are laid down anatomically, which might insure any clear-cut specificity of function to special subdivisions of this system The fact that the orbito-temporal portion of the limbic system on stimulation seems to activate more readily "oral" mechanisms involved in alimentary activities whereas the cingulate-hippocampal part seems to be more closely related to the production of "attentive" behavior is not in serious contradiction to this rule, since a very wide overlap of both types of responses is obvious from all stimulation studies. That some reservations should be made in this respect concerning the functions of the hippocampus proper has been pointed out before

A closer understanding of the functional relationship between the limbic system and the hypothalamus and its immediate caudal continuation into the rostral mesencephalic tegmentum can be obtained by studying the projection pattern of limbic structures upon these subcortical formations In our own laboratory we have subjected the amygdala to a thorough electrophysiological investigation of its projection pattern (Gloor, 1955), and we believe that our findings may shed some light upon the problems just raised The amygdala and its subcortical projection pattern may be taken as a good example illustrating the general relationship of limbic structures to the subcortex, since in physiological properties it partakes of the functional patterns of the orbito-temporal as well as of the cingulate-hippocampal type of activities, in other words, in stimulation studies it produces "oral" types of behavior as well as the pure "attentive" pattern merging into fear and rage reactions (Kaada, 1951, Gastaut, 1952, MacLean and Delgado, 1953, Kaada, Andersen and Jansen, 1954).

place over the short elements of the medial forebrain bundle system, which in its more cephalic course clearly shows short latency responses to amygdaloid stimulation (Figure 4A). Naturally, such a system, by means of its multi-synaptic structure, is highly capable of integrating complex patterns of activities.

Also of interest, in view of our present subject, is the fact that the amygdala is indirectly connected with the hippocampus over multi-synaptic relays running most probably through the piriform lobe Long latency responses to amygdaloid stimulation are regularly recorded in the hippocampus. This relationship is interesting for the hippocampus via the fornix in turn influences the same general subcortical region centered upon the hypothalamus.

Amygdaloid Stimulation: Baso-lateral Subdivision

(B) Long Latency Responses

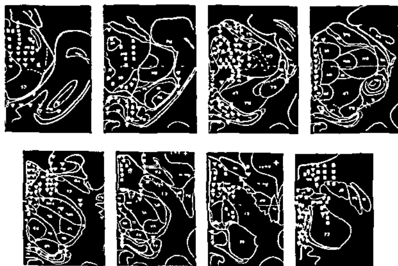
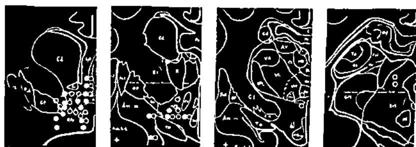


Figure 4B Long latency responses from stimulation of basolateral amygdala The cross indicates the area stimulated Symbols filled squares 10-14 msec latency, filled triangles 15-20 msec latency, empty squares 21-25
measure-
(From

Amygdaloid Stimulation Baso-lateral Subdivision

(A) Short Latency Responses



Amygdaloid Stimulation Cortico-medial Subdivision

(A) Short Latency Responses



Figure 4A Map of distribution of short latency responses from stimulation of basolateral and corticomedial subdivision of amygdala Stimulation point indicated by cross Symbols filled circles latency shorter than 7 msec, empty circles 7-9 msec latency (All measurements taken from the beginning of the 5 msec stimulus artefact) (From Gloor *EEG Clin Neurophysiol*, 1955)

mesencephalic tegmentum, including the whole hypothalamus, the subthalamus, and parts of the diffuse projection system of the thalamus. The most prominent responses are obtained in the infundibular part of the hypothalamus, in and around the ventromedial nucleus (Figure 5) The long latency of the responses suggest that transmission in this subcortical system takes place in multisynaptic relays made up of short neurons. The mediation of the responses over this multi-synaptic network probably takes

Moreover, it has become apparent in our experiments that the baso-lateral amygdala, although provided with a direct pathway to the subcortex, also mediates responses which are synaptically and eventually even multi-synaptically, relayed through the cortico-medial amygdaloid nuclei, from where efferent fibers again run to the septo-hypothalamic region, this time via the stria terminalis.

Therefore, it seems that a rather complex projection pattern exists connecting the amygdala to the hypothalamus and to adjacent subcortical gray matter, with provision for direct and indirect discharge paths, some involving the hippocampus and fornix after multi-synaptic relays in the piriform lobe (Figure 6). That the secondary indirect hippocampal route is more than just accessory is evidenced by the fact that the hippocampal potentials obtained by amygdaloid stimulation build up to an extremely high voltage when recruited with repetitive amygdaloid stimulation (Figure 7) (Gloor, 1955).

Through these various routes the amygdala is therefore able to influence the whole central basal gray matter extending from the septum through the hypothalamus back to the mesencephalic tegmentum This area has been shown to integrate most important autonomic, somatic, behavioral and endocrine mechanisms (Hess, 1949, 1954; Magoun, 1950, Penfield, 1950, Harris, 1951).

The functional significance of this relationship becomes clear when we recall the results of electrical stimulation of these septohypothalamic and adjacent subcortical regions to which the amygdala projects (Hess, 1949, 1954; Magoun, 1950, and others). When we compare them with the responses obtained from the amygdala, we realize that all responses produced by amygdaloid stimulation are also elicited in one or the other of the subcortical gray formations to which the amygdala projects (Figure 8) In contradistinction, however, to the functional representation in the amygdala there is here in the hypothalamus and in the adjacent subcortical areas a clear topographically organized grouping of those single responses, which are components of a common purposeful global response These purposeful functional patterns are represented separately in more or less well defined topographical fields (Hess, 1949, 1954) Such an organization is absent in the

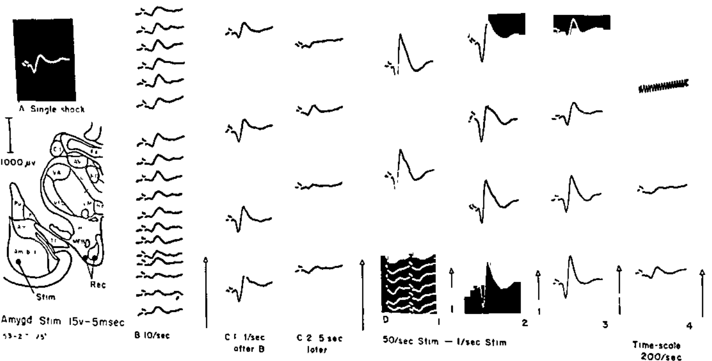


Figure 5 Response in the ventromedial nucleus of hypothalamus

A single shock response
B. response during 10 c/sec repetitive amygdaloid stimulation, no recruiting
C potentiation induced by 10 c/sec repetitive amygdaloid stimulation.
D end of a train of 50 c/sec repetitive amygdaloid stimulation with consecutive potentiation (From Gloor *EEG Clin Neurophysiol*, 1955)

the amygdala. Their results conflict somewhat with each other and in turn are contradictory to findings from other laboratories (Gastaut, 1952, MacLean and Delgado, 1953). This seems to be rather in favor of a non-topographical type of representation in the amygdala

The projection pattern of the amygdala as revealed by our electrophysiological studies makes it easier to understand why such a wide variety of apparently quite unrelated somato-autonomic mechanisms can be activated by amygdaloid stimulation. Excitation arising in the amygdala can spread rather widely within a large subcortical area known to govern, in its different constituent parts, all the different activities upon which amyg-





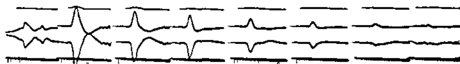


Figure 7 Hippocampal response to repetitive amygdaloid stimulation A. recruitment during repetitive amygdaloid stimulation at ten c/sec Note decrease in latency during recruitment B potentiation of single shock response after end of repetitive stimulation Note that gains in B are lower than in A Second response in B is about the same size as the response at the end of repetitive stimulation in A

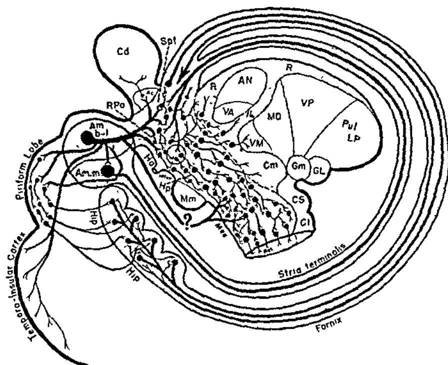


Figure 6 Diagram of the neuronal organization of the amygdaloid projection system as revealed by electrophysiological studies. Ac nucleus accumbens Am b-l. basolateral subdivision of amygdala Am m corticomedial subdivision of amygdala AN. anterior thalamic nuclei. Cd caudate nucleus CI inferior colliculus Cm centre median. CS superior colliculus GL lateral geniculate body Gm medial geniculate body Ha anterior hypothalamus Hip hippocampus Hp posterior hypothalamus IL intralaminar nuclei of thalamus LP nucleus lateralis posterior thalami MD nucleus medialis dorsalis thalami Mes mesencephalon Mm mammillary body NHvm nucleus ventromedialis hypothalami Pul pulvinar R nucleus reticularis thalami Ret reticular formation RPo regio preoptica Spt septum. VA nucleus ventralis anterior thalami VM nucleus ventralis medalis thalami VP nucleus ventralis posterior thalami. The dotted area represents the subcortical integrative areas regulating "global" mechanisms and the limbic structures projecting into it. (From Gloor *EEG Clin Neurophysiol*, 1955)

amygdala, as it is in other parts of the limbic system We feel justified in maintaining this position in spite of recent attempts by Kaada, *et al* (1954) and Koikegami, *et al.* (1952, 1954) to arrive at a concept of topographical organization of functions in

the amygdala. Their results conflict somewhat with each other and in turn are contradictory to findings from other laboratories (Gastaut, 1952; MacLean and Delgado, 1953). This seems to be rather in favor of a non-topographical type of representation in the amygdala.

The projection pattern of the amygdala as revealed by our electrophysiological studies makes it easier to understand why such a wide variety of apparently quite unrelated somato-autonomic mechanisms can be activated by amygdaloid stimulation Excitation arising in the amygdala can spread rather widely within a large subcortical area known to govern, in its different constituent parts, all the different activities upon which amyg-

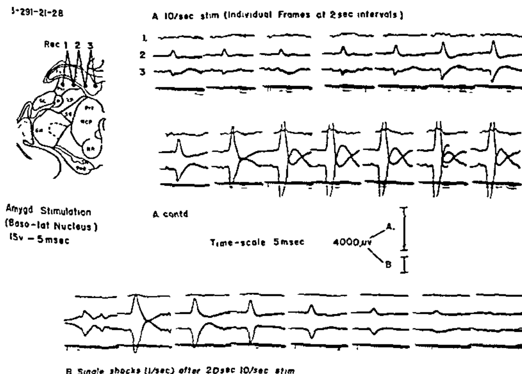


Figure 7 Hippocampal response to repetitive amygdaloid stimulation. A recruitment during repetitive amygdaloid stimulation at ten c/sec. Note decrease in latency during recruitment. B potentiation of single shock response after end of repetitive stimulation. Note that gains in B are lower than in A Second response in B is about the same size as the response at the end of repetitive stimulation in A.

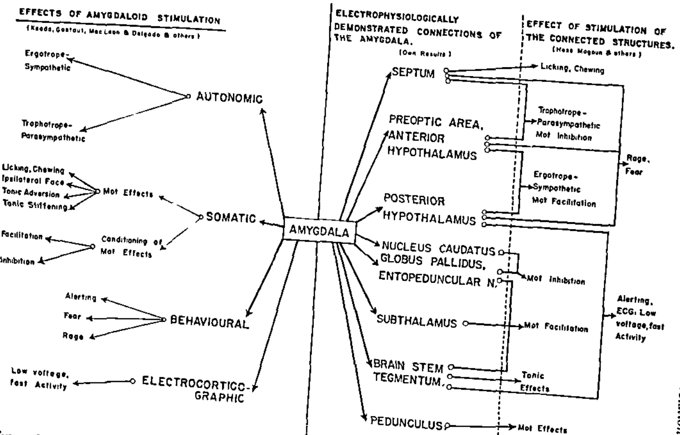


Figure 8 Diagram illustrating the electro-physiologically demonstrated direct and indirect connections of the amygdala and their functional significance. On the left hand side are listed the responses to amygdaloid stimulation as described in the literature. The middle column lists the structures which on electrophysiological investigation were shown to be fired in a direct or indirect way from the amygdala On the right hand side the responses obtained by stimulation of these structures as reported by Hess, Magoun and others are listed. Note that the responses obtained on amygdaloid stimulation are also obtained from one or other of the subcortical structures to which the amygdala projects and that there is a topographical grouping of these effects in the subcortex, and that such a grouping is absent at the amygdaloid level (From Gloor *EEG Clin. Neurophysiol.*, 1955.)

daloid stimulation has been shown capable of exerting its influence. This brings the mechanism by which these responses are mediated closer to our understanding, their true functional significance within the framework of organismic functions as a whole, however, still remains obscure at this stage.

ABLATION STUDIES

In order to arrive at a better understanding of the functional significance of the limbic system, confrontation of the stimulation results with those obtained by ablations is most important. In our opinion it is this comparison which may give us the essential key to the understanding of the functional role of this system.

The first and most important conclusion one may draw from all ablation studies performed in the limbic system is that these lesions do not interfere with the correct integration of basic autonomic and somato-motor mechanisms, in spite of the fact that the removed areas have been proved to exert active influences upon these very same functions which fail to be disturbed after removal of the active area. This is not only true for unilateral, but as well for bilateral symmetrical and extensive lesions This proves that the limbic system cannot be essential for the integration of homeostatic and basic adaptive autonomic functions and for the correct performance of somato-motor activities This integration depends entirely upon hypothalamic and brain stem mechanisms. This somewhat unessential character of limbic function makes it also easier to accept the notion of the unspecificity of autonomic and somatic representation in the limbic system, as revealed by stimulation studies.

The changes induced by bilateral ablations of limbic structures, although not interfering with organismic functions primarily essential for survival, nevertheless produce important behavioral changes involving the affective and sexual behavior of animals subjected to such lesions (Klüver and Bucy, 1937, 1938, 1939, Spiegel, Millner and Oppenheimer, 1940; Bard and Mountcastle, 1948, Smith, 1945, Ward, 1948; Ruch and Shenkin, 1943, Livingston, *et al*, 1948; Glees, Cole, Whitty and Cairns, 1950, Rosvold, 1951; Pribram and Bagshaw, 1953, Schreiner and Kling, 1953; Pribram and Fulton, 1954). The basic change seems to be

one towards "tameness," sometimes associated, in mesial temporal lesions, with hypersexuality. These results of ablation studies can briefly be summarized as follows:

(1) The most striking change in behavior encountered after *removal of the anterior cingulate, orbito-temporal or amygdaloid* portion of this region consists of a marked diminution of fear and rage responses and an absent or diminished avoidance of situations exposing the animal to painful or frustrating experiences. It should be mentioned here, however, that bilateral amygdaloid *removals have also produced opposite results in cats* as reported by Spiegel, Millner, and Oppenheimer (1940) and by Bard and Mountcastle (1948) These authors obtained lowered rage thresholds. Their animals displayed a savage behavior This discrepancy is difficult to explain. In view of more recent experimental work carried out in different animal species, including primates, rodents, cats and other carnivores, it seems, however, highly probable that the basic change produced by such removals is one toward tameness rather than the opposite

(2) Increased motor restlessness ensues after anterior cingulate and orbito-frontal removals It is, however, sometimes also seen in the type of removal just described and resulting in tameness. On the other hand, these changes could not be confirmed in similar lesions carried out in man (Scoville, 1949, Scoville, *et al*, 1951, LeBeau, 1951)

(3) Mesial temporal removals, including the amygdala, in *addition to tameness, produce a curious oral compulsive behavior.* Animals with such lesions show a strong urge to put all objects, even burning matches, in their mouth Together with this there is an intense release of sexual drive resulting in an abnormally exaggerated sexual behavior (Kluver and Bucy, 1937, 1938, 1939, Schreiner and Kling, 1953) Castration studies in these hypersexual animals suggest that this hypersexuality is the result of a release of sex hormone production probably mediated over the hypothalamo-pituitary system (Schreiner and Kling, 1954)

(4) Hippocampal lesions are conspicuously ineffective and produce almost no changes at all Pleasure reactions to petting may eventually be facilitated by such lesions (Bard and Mountcastle, 1948).

The final conclusion from these ablation studies is that apparently the limbic system is not critically involved in the integration of the very same somato-autonomic mechanism which it is apt to influence on electrical stimulation and that the main effects of ablations suggest that these areas are somehow concerned with the integration of affective and sexual behavior. It is therefore apparent that the central and essential integration of autonomic and basic somatic functional patterns takes place at the hypothalamic and tegmental level. Similar views to this are expressed by Hess (1954) and his co-workers (Hess, Akert and McDonald, 1951; Hess, Akert and McDonald, 1952). If the limbic system is not concerned with this basic integration, some other function must be attributed to it.

THE DYNAMIC ELECTROPHYSIOLOGICAL PROPERTIES OF THE AMYGDALOID PROJECTION SYSTEM AND THEIR POSSIBLE FUNCTIONAL SIGNIFICANCE

Our recent electrophysiological studies of the amygdaloid projection system (Gloor, 1955) are interesting in this context inasmuch as they may help us to understand the functional significance of the peculiar relationship between the limbic system and the hypothalamus.

These investigations on the amygdala were carried out with a stimulation technique using first single shock stimulation, followed by repetitive stimulation, which, in turn, was immediately followed by single shock stimulation, thus allowing observation of after-effects of repetitive stimulation on the responses recorded in the amygdaloid projection field The rate of repetitive stimulation was 10 or 50 c/sec. and usually was applied for a duration of 10 sec.

Investigating the amygdaloid projection system with this method it was seen that the electrical responses to amygdaloid stimulation, as recorded in its projection field, are not rigidly determined, but vary with the rate of stimulation and that considerable changes in the excitatory state occur during repetitive stimulation and often outlast the discontinuation of repetitive firing for a relatively long time.

These changes induced by repetitive stimulation were of three orders

(1) Recruiting, or more rarely the opposite, progressive obliteration, of the response during repetitive stimulation. Recruiting was most prominent in the hippocampus where the recruited response could finally reach an amplitude ten times that of the initial single shock response (Figure 7). Fairly prominent recruiting was also seen in the rostral brain stem tegmentum.

In the anterior hypothalamus, however, e.g., in the ventromedial nucleus, there was usually no recruiting, but rather a tendency to obliteration (Figure 5).

(2) Potentiation was a very common effect of repetitive amygdaloid stimulation. This term serves to describe the phenomenon, that a response to single shocks applied immediately after the end of repetitive stimulation is of higher voltage than the response to single shocks delivered before repetitive stimulation This state of potentiation decays over a period of a few seconds, the degree and duration of potentiation at a given location being dependent upon the rate and duration of the preceding repetitive firing. This indicates the presence of a slowly decaying state of a heightened central excitation Potentiation may quite naturally follow after recruitment on discontinuation of repetitive and resumption of single shock stimulation, as it is the rule for the hippocampus (Figure 7) and the rostral mesencephalic tegmentum However, potentiation does not necessarily depend upon preceding recruitment, since it can occur without recruitment or even after obliteration, as seen, e g., in the ventro-medial nucleus of the hypothalamus (Figure 5) or in the posterior hypothalamus (Figure 9)

(3) Changes in latency, either an increase or a decrease, going along with recruitment and potentiation were also seen (Figures 5, 7, and 10). They probably depend upon complex synaptic processes occurring in multi-synaptic systems

All these changes were most prominent and more apt to occur in multi-synaptically relayed responses and were practically absent from the primary projection area of the amygdala as, e g, in the lateral preoptic and adjacent anterior lateral hypothalamic area, which receives direct afferents from the amygdala. Here

the responses were very constant. No recruiting and no latency changes and only a minimal potentiation were observed (Figure 11).

This indicates that under amygdaloid stimulation the excitatory state in the multi-synaptic septo-hypothalamo-tegmental core of grey matter is very flexible and subject to important fluctuations according to the rate and duration of firing Furthermore, in addition to direct amygdalo-subcortical discharges, there is the very important multi-synaptic indirect discharge path over the hippocampus and fornix which partly, at least, fires the same subcortical structures, and, which due to its slow but very powerful recruiting characteristics, will progressively modify its contribution to the elaboration of an integrated subcortical response dur-

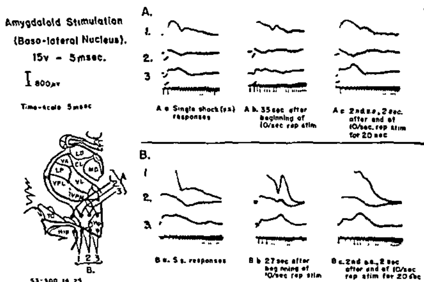


Figure 9. Responses in the posterior hypothalamus on stimulation of the basolateral amygdala Responses in channel 1 in B are from the hippocampus. Channel 3 response is from the posterior hypothalamus, more dorsally in A than in B Note that the single shock response ...

... response, as evidenced by its latency) After discontinuation of repetitive stimulation, there is potentiation of single shock responses in the posterior hypothalamus

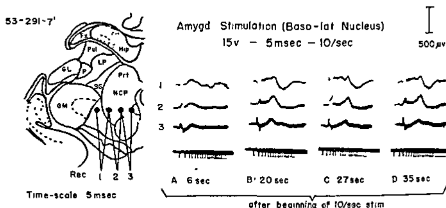


Figure 10 Repetitive amygdaloid stimulation at 10 c/sec · recruiting responses in the nucleus commissurae posterioris at the junction between diencephalon and mesencephalic tegmentum Note the considerable increase in latency of the response during recruitment. (From Gloor *EEG. Clin Neurophysiol*, 1955.)

ing the course, and for a while after cessation, of repetitive firing from the amygdala. The amount of hippocampal interference will also certainly be different at slow or at high rates of amygdaloid firing

It therefore appears that there are many possibilities of modulating a response by the nature of the mechanisms through which the amygdala fires into its subcortical projection field This relationship is interesting in view of the fact that so many autonomic and somatic activities of the hypothalamo-tegmental region can be reproduced from the amygdala—often in quite a temperamental way—and that these very activities remain basically undisturbed after bilateral removal of the amygdala This suggests, in the light of the demonstrated physiological properties of this system, that the amygdala exerts a rather subtle, modulatory influence upon these septo-hypothalamic and tegmental areas and that it is not in actual command of the mechanisms which are dependent upon these structures Corroborating evidence for this is given by Naquet (1953), who, when reviewing the results of amygdaloid stimulation carried out in Gastaut's laboratory, states that noticeable autonomic, somatic and behavioral effects fail to occur, unless there is afterdischarge, i e, seizure

activity in the amygdala Hess and his collaborators (1951, 1952, 1954) have also pointed out that cortical influences exerted upon autonomic functions are of a somewhat unessential order and have emphasized the central position of the hypothalamus in the over-all integration of autonomic functions

This strongly suggests that under normal conditions the amygdala and probably the whole limbic system is not, or only exceptionally, capable of actively organizing somato-autonomic or behavioral patterns of function, but its activity must be more discrete and probably of a modulatory character. The electrophysiologically determined projection pattern of the amygdala and the dynamic properties of this projection system are well capable of such a type of activity. The multi-synaptic system to

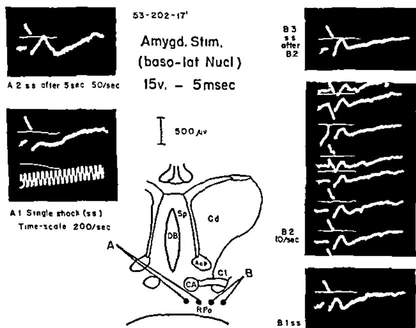


Figure 11 Amygdaloid stimulation. response in preoptic region A medial preoptic area, small response potentiated after repetitive amygdaloid stimulation B lateral preoptic area Short latency response remaining stable during repetitive stimulation and showing only minimal potentiation to single shocks after discontinuation of repetitive stimulation (From Gloor *EEG. Clin Neurophysiol*, 1955)

which the amygdala projects has all the characteristics required to act as a flexible integrator, since it provides for multi-synaptic contacts in a common neuronal pool shared by other neuronal systems.

CONCLUSIONS

On the basis of these data we may try to arrive at a functional concept describing the role of the limbic system as a modulator of patterns of nervous activities integrated at the level of the hypothalamus and its continuation into the mesencephalic tegmentum.

In this, the limbic system may act as an important link between the neocortex and the hypothalamo-mesencephalic gray matter. The functional significance of this relationship may be better understood in evolutional terms In lower vertebrates the behavior of the organism is organized in rather simple and fixed patterns integrated in the hypothalamo-tegmental system, regulating, as well, the homeostatic functions of the organisms as its adaptational reactions These patterns are essential for survival and remain so even after the acquisition of neocortex in mammals, which allows the development of an increasingly "free" and "extrovert" behavior, the more the neocortex grows in importance. This new type of behavior is largely dependent upon learning and discriminative intelligence and is able to organize highly differentiated and skillful motor patterns It cannot, however, free itself completely from the basic, archaic mechanisms, integrated in the subcortex, whose functional patterns represent the essential background upon which neocortical function has to evolve. How much neocortical activity is dependent upon these subcortical mechanisms is evidenced by the fact that consciousness—a basic prerequisite for neocortical functions—is entirely dependent upon their integrity The hypothalamo-tegmental system still remains the highest integrator of the homeostatic and basic somatic and behavioral functional patterns creating the adequate background upon which the neocortex can play

To make full use of these old subcortical mechanisms the neocortex has to exert some flexible influence upon them This influence has to be mediated through a system which, as the limbic system, is provided with rich connections to the hypo-

thalamo-tegmental area and whose functional properties allow such a flexible play.

It has been stated many times by several authors (Papez, 1937; MacLean, 1949, 1952, 1954; Fulton, 1951; Gastaut, 1952) that the limbic system is concerned with the elaboration of emotional expression. This may well be one of its chief functions, since emotional expression conceivably acts through the just postulated modulatory mechanisms and leads to somato-autonomic changes, which are somewhat reminiscent of those seen in limbic stimulations This theory would also well fit into the concept that the limbic system is somehow a mediating link between the neocortex and the older parts of the diencephalon There can be no doubt, indeed, that at least in man and in most mammals emotion is dependent upon the neocortical analyzer since often the emotional content of a situation cannot be "recognized" as such, unless it is analyzed by a discriminative process dependent upon neocortical function The emotional response which follows could then be mediated through the limbic system to somato-autonomic effector mechanisms of the subcortex, especially of the hypothalamus, which even without any cortex is still able to integrate quite by itself the fully organized effector mechanism of such emotions at least as rage and fear (Bard and Mountcastle, 1948 Compare also with studies done by Hess, 1949, 1954, and Hess and Bruegger, 1951).

It is, however, quite conceivable that the modulating influence of the limbic system is not exclusively concerned with the mediation of emotional expression but also acts in correlating appropriate homeostatic and adaptive mechanisms to neocortically directed activities in general as, e.g., during voluntary physical work or during intense intellectual concentration, or when we voluntarily induce ourselves to relax and so on

All of what has been said so far has apparently very little to do with neuro-endocrine regulations I do not think, however, that in a broader sense this is necessarily true. From the general character of the influences exerted upon the hypothalamus by the telencephalon it certainly appears unlikely that one will find at any cortical level, not even in the limbic system, any fundamental integration of neuro-endocrine mechanisms, but it is quite possi-

ble and even likely that modulatory effects upon the endocrine system may play a role in limbic function as well. In view of the possible importance of this old telencephalic system for the integration of affective behavior, it will be of great interest to study the relations of limbic physiology to the problem of emotional stress. This subject is still wide open to investigation, and if this review has helped to set the stage for such work, its modest purpose has been fulfilled.

SUMMARY

(1) Anatomical studies of the telencephalo-hypothalamic connections reveal that the hypothalamus receives its afferents mainly from the oldest parts of the cortex and the basal ganglia. These structures comprise the archicortex, i.e., the hippocampus, the paleocortex, i.e., the cortex of the piriform lobe, the mesocortex, i.e., the cortex of the cingular gyrus, the anterior insula and of the posterior orbito-frontal region, the archistriatum, i.e., the amygdala, and the paleostriatum, i.e., the globus pallidus. With the exception of the globus pallidus, all these structures have been incorporated in what has been called the limbic system. Each portion of this system by various routes sends fibers into the medial forebrain bundle of the hypothalamus in addition to more specific projection patterns upon various hypothalamic nuclei.

(2) Stimulation within the limbic system produces a wide variety of autonomic (sympathetic and parasympathetic), somato-motor, electrocorticographic, endocrine, and behavioral responses. The principle of topographical organization of specific effects into specific anatomical fields is, however, not realized in the limbic system. There is a wide overlap of all sorts of responses of even antagonistic character (e.g., increase and fall in arterial pressure, facilitation and inhibition of evoked motor activities), and although this system may not be truly equipotential throughout its extent—reservations in this respect especially apply to the hippocampus—it appears that all constituent parts of the limbic system subserve similar functional mechanisms.

(3) The projection pattern of the amygdala is taken as a representative example to illustrate the underlying anatomical rela-

tionship of such a diffusely active system. Electrophysiological studies show that the amygdala is able to activate a widespread subcortical field extending from the septum through the hypothalamus to the tegmentum mesencephali, a region, which, in turn, has been shown to integrate in its different constituent parts specific groups of functions activated by amygdaloid stimulation. The difference between the limbic system and the subcortex is that in the subcortex there is a clear topographical grouping of certain effects subserving a common functional purpose, whereas such a topographical organization is apparently absent in the limbic system.

(4) Bilateral ablations of limbic structures show that such lesions do not interfere with the correct performance of somatomotor activities nor do they permanently upset the central integration of the basic homeostatic and adaptive autonomic mechanisms despite the fact that these very same limbic structures are quite capable of influencing these mechanisms on stimulation On the other hand, bilateral limbic lesions produce alterations of behavioral mechanisms involved in affective and sexual activities

(5) The electrophysiological properties of the amygdaloid projection system demonstrate that there is a wide range of flexibility in the excitatory state of the neuronal systems, which the amygdala is capable of influencing Studies with repetitive amygdaloid stimulation produced prominent recruiting and potentiation phenomena in the amygdaloid projection fields These mechanisms may play an important role in modulating subcortical activities

(6) The hypothesis is advanced that the limbic system does not fundamentally integrate the functions it is capable of influencing by its activity, but rather acts as a modulator of functional patterns integrated at the level of the hypothalamus and the brain stem tegmentum It may represent an important link between neocortex and hypothalamo-tegmental formations conveying information enabling the subcortically induced activities to adapt to the patterns organized by the neocortex Such adaptational mechanisms may be involved in emotional expression It is finally suggested that the limbic system may take part in activation of the correlated endocrine mechanisms

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STEROID HORMONAL RESPONSE TO STIMULATION OF ELECTRODES IMPLANTED IN THE SUBFRONTAL PARTS OF THE BRAIN

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The steroid hormonal substances have been implicated both in the normal physiological state and in many diseases and syndromes. Alterations in the quantity of these compounds excreted in urine have provided the basis for most clinical-laboratory studies in this field up to date, but more recently it has been possible to extract and study these steroids in whole blood [1, 2, 3, 4]

The beginnings of modern hormonal chemotherapy started about fifteen years ago with the first deliberate castrations for advanced prostatic cancer [5]

Although the 17-ketosteroids in urine constitute almost the only portion of steroid metabolism which was studied on a regular basis until five years ago, it has been known since the early work of Reichstein in 1936 that an entirely different group of steroid substances, the "corticoids," are produced in large amounts in the human organism and participate in an essential way in the maintenance of body homeostasis. Modern hormonal chemical techniques permit the direct assay of corticoid substances such as Kendall's Compound E (cortisone) and Compound F (hydrocortisone), as well as Compound B (corticosterone) Most recently the work of Selye has brought into sharper focus the importance of the corticosteroids in the over-all picture which is frequently called "the stress syndrome"

Recently developed techniques for implantation of electrodes and stimulation of the deep subfrontal parts of the brain have

relieved intractable pain due to cancer and arthritis according to Heath, *et al*, of Tulane [6]

In our six patients so treated, electrodes were implanted in the subfrontal parts of the brain and stimulation through these electrodes was instituted in an attempt to control the distressing pain of their malignant disease. In an attempt to document what occurs within the patient following this kind of stimulation, the steroid hormonal responses have been measured For this purpose, both blood and urine have been extracted and studied for 17-ketosteroid content and for corticosteroid content. These direct chemical analyses have been chosen in preference to the more commonly employed, but less reliable, techniques such as the eosinophile response, lymphocytic response, ascorbic acid content of the adrenal gland and the measurement of ACTH in the circulating blood, the last two obviously being impossible in the intact patient. The technique of Sayers for assaying, by biological means, the amount of circulating ACTH is useful only in unusual situations, like Addison's disease.

CLINICAL STUDY

Six patients, all but one of whom had constant severe pain, were chosen for treatment, and the patients or their families all agreed to this procedure after full explanation. The operation was designed to place electrodes in the brain anterior and ventral to the anterior commissure within 1 cm of the midline in the region of the septal nuclei as indicated by Heath and his Tulane Group by a stereotaxic apparatus under x-ray control Subsequent stimulation was performed in an identical manner on every patient, using a current of 4 milliamperes or more As indicated by postoperative x-rays and two autopsies, the electrodes were by no means in exactly corresponding places in all patients.

The physiological changes that occurred during stimulation in three patients consisted of alterations in blood pressure, pupillary dilatation, and in one case, piloerection Two patients felt "good" during stimulation, one drowsy, and this one once experienced a rage reaction, when the electrodes close to the anterior commissure were activated (Autopsy verification of electrode placement was obtained in this case)

Mock stimulations were performed by having the equipment and personnel deployed as for a real stimulation but with the patient unaware that no electrical current was being applied. None of these mock stimulations produced detectable therapeutic or observed physiologic changes. Parenthetically it should be stated that no patient died as the result of electrode implantations or suffered any complications from this procedure.

Patient No 1 (G) was a sixty-one-year-old white woman who had severe pain due to amyloidosis and multiple myeloma. She required 600 mg. of demerol per day prior to stimulation; after stimulation only 25 to 60 mg. per day for three weeks time. Transient polydipsia, polyuria, and a urine of very low specific gravity followed the second stimulation for three days, suggestive of temporary diabetes insipidus, and pain was again relieved for another three weeks. This was the only patient to show an increased output of blood and urinary steroids after stimulation, possibly because she was the only one in whom the electrodes were close to the anterior aspect of the hypothalamus.

Patient No 2 (P) was a thirty-seven-year-old woman with disseminated leio-myosarcoma of the uterus causing pain in the low back, chest, and upper abdomen The first two stimulations resulted in improvement, but not disappearance of pain, lasting for five to six days only Later stimulations were not followed by improvement, attributed perhaps to a break in one electrode wire later found at autopsy.

Patient No 3 (K) was a fifty-six-year-old woman who had been confined for years to a state mental hospital for paranoid schizophrenia Despite a radical mastectomy for mammary carcinoma, the tumor had metastasized to the spine, pelvis, and skull. The patient was treated by bilateral ovariectomy and adrenalectomy subsequent to subfrontal implantation of electrodes. After stimulation and operation, the patient became tolerant of her family for the first time in years and did not complain of pain However, she did not complain of pain prior to operation Following stimulation at operation this patient showed an apparent decreased output of blood corticosteroids in both the circulating blood of the femoral and adrenal veins cannulated at operation

Patient No 4 (M) was a twenty-seven-year-old male suffering from extreme epigastric, lower thoracic, and supraclavicular pain produced by an incurable metastatic carcinoma of the stomach

Stimulation of the electrodes in this patient on one occasion produced a seizure, the electrode used for this particular stimulation being found at autopsy to lie in the right internal capsule. However, no relief of pain resulted from stimulation of any pair of electrodes. During one long stimulation, lasting forty-five minutes, the patient became drowsy but would always respond immediately and coherently to verbal stimulations A change in current density in either direction would also rouse him temporarily His original pain persisted during the periods of wakefulness Stimulation on one other occasion resulted in a rage reaction with behavior quite out of character for this patient, but these rage reactions subsided immediately when the stimulation was discontinued The patient was quite aware of this behavior, but was unable to explain or control it Autopsy showed one electrode below the anterior commissure in caudate nucleus, the other above the anterior commissure in caudate nucleus

Patient No 5 (C) was a seventy-year-old woman suffering from left-sided burning paresthesia (thalamic syndrome) due to a cerebral vascular accident eight years previously. Before this she had undergone a bilateral thoracolumbar sympathectomy for vascular hypertension. Stimulation of the electrodes in this patient, which were deliberately placed in the midportion of the frontal white matter, did not relieve her symptoms.

Patient No 6 (T) was a fifty-nine-year-old laborer who had severe constant pain in the chest, low back, and left arm and shoulder caused by a massive mesenthelioma of the pleura Stimulation of the implanted electrodes afforded no relief However, extensive steroid studies performed on him revealed an apparent decrease after stimulation. While autopsy permission was not obtained, we believe the electrodes were in the gyrus rectus

BIOCHEMICAL ASSAY FOR STEROIDS

Standard methods were used for the study of both blood and urine The 17-ketosteroids are estimated solely by the Zimmerman reaction; there is a double check on urinary corticoids obtained by using the blue tetrazolium reaction and by the formaldehydogenic steroid estimation [7]

Although many of the same principles employed for urinalysis are also used for blood, certain differences in extraction and separation techniques were necessary Usually, isolated Compound F

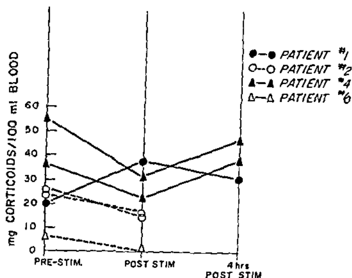


Figure 1.

(hydrocortisone) was measured and considered the corticoid of essential importance.

Our first studies on the effects upon total circulating blood corticoids as a result of stimulation of implanted electrodes are summarized in Figure 1 It is obvious that in Patient No. 1 there is a 100 per cent *increase* in circulating corticoids, but that in every other case the blood value is depressed by stimulation Repetition of the experiment in Patients No 2 and 4 gives good duplication of the apparent depressant effect initially observed It is pertinent to note that the position of electrodes in Patient No. 1 was closer to the anterior hypothalamus than in any other patient, which may account for the increased output of blood corticoids after stimulation in this patient and not the others

In more recent studies, we have studied somewhat more extensively the urinary excretion of steroids as well as steroid blood levels. In addition, the normal diurnal variation in these values has been determined prior to the performance of both mock stimulations and actual stimulations

A presentation in detail of one case studied in this way may prove interesting In Figure 2 are charted the 17-ketosteroid values in the urine of this patient The broken-line graph with the triangular symbol shows the patient's diurnal variation, in 6-hour

pooled collections, in output of urinary 17-ketosteroids. The solid-line graph depicts the rise in the urinary values after mock stimulation. The depression and subsequent "rebound" following actual stimulation is shown in the dash-lined, hollow-square graph. In all of the charts on this patient, both mock and actual stimulations were done after the second charted value on the graphs.

In Figure 3 for the same patient the urinary corticoids as estimated by the blue tetrazolium reaction are plotted. Here again the expected decrease during the day as a normal variant is encountered. As with 17-ketosteroids, the corticoids in urine are elevated by mock stimulation and temporarily depressed by actual stimulation of electrodes.

These findings are corroborated by the determination of formaldehydogenic steroids in the urine as shown in Figure 4. The

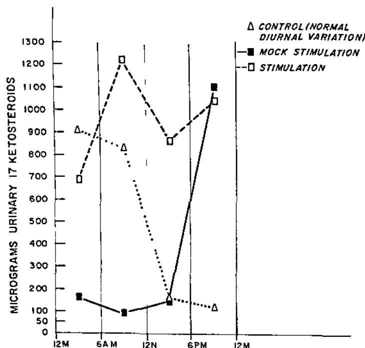


Figure 2.

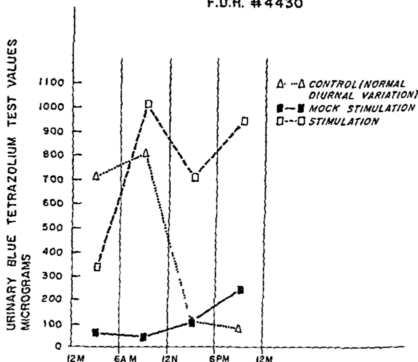


Figure 3

values obtained on the day of actual stimulation could not be included in the scale used here, therefore the numbers have simply been written into the chart beside the graph. This chart completed the urinary study for this patient which shows a consistent increase in both 17-ketosteroids and corticoids in urine with mock stimulation, and a consistent *decrease* with *actual* stimulation

One further set of biochemical observations was made in this individual. These are summarized in Figure 5 indicating the quantity of Compound F (hydrocortisone) in circulating blood at different times during a usual hospital day and during a day in which mock stimulation was performed, and finally during a day in which actual electrode stimulation was performed The diurnal variation is seen to follow the trend of corticoids in urine. Mock stimulation (the solid-line graph) produces a significant increase in blood hydrocortisone levels, and actual stimulation a decrease

The time of mock and actual stimulations is after the second charted blood level on each graph.

A final study which may lend significance to the foregoing data merits brief mention. This study on Patient No 3 was done during operation at the time that the second (left) adrenal gland was to be removed (several days after the implantation of electrodes) and subsequent to bilateral ovariectomy and right adrenalectomy at this operation (Figure 6). Presumably therefore the left adrenal gland at this time was the only remaining source of endogenous corticoids. Note the changing level of circulating blood corticoids as the patient is followed through medication,

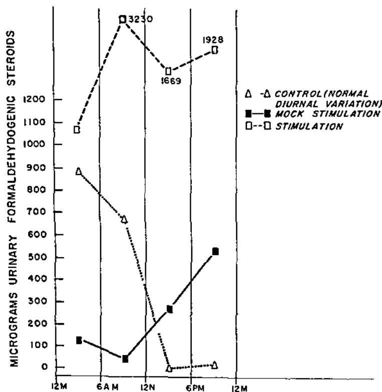


Figure 4

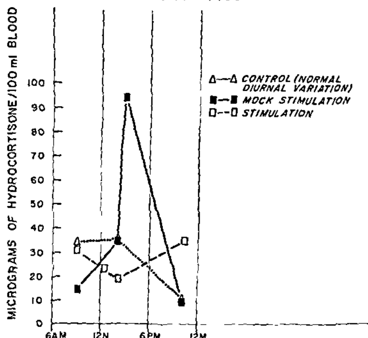


Figure 5.

anesthesia and the progress of adrenal surgery. At 11.00 a m, with the femoral and left adrenal veins cannulated, final base-line corticoid levels were determined It is seen that stimulation of the electrodes in the brain then produce an apparent decrease in the rate at which the left adrenal is producing corticoids and pouring them into the circulation

SUMMARY

(1) From these preliminary studies it appears that stimulation of the brain close to the anterior hypothalamus may lead to an increased output of blood and urinary steroids, a significant alleviation of pain for three weeks time, and physiological changes at the time of stimulation, along with an immediate sense of well being at the onset of stimulation These phenomena could be repeated on subsequent stimulations with the same electrodes.

(2) On the other hand, stimulation of electrodes in the more anterior, inferior, or lateral position to those of Patient No 1

(above) seemed less effective as to pain relief and physiological responses, and apparently resulted in a *reduction* rather than an increase in blood and urinary steroid levels. This reduction was also noted (following stimulation) in blood obtained directly from the adrenal vein It has been shown that the output from the adrenal gland remains constant unless some type of systemic or local stimulus is applied In this case a decrease in adrenal corticosteroids occurred We feel that this points to a direct effect of central origin upon the adrenal gland.

(3) We cannot be sure that electrodes were in the so-called septal area in any patients and are certain they were not in two.

(4) The findings suggest the possibility of specific activating and perhaps depressing pathways for steroid production.

(5) We feel further studies along these lines are warranted

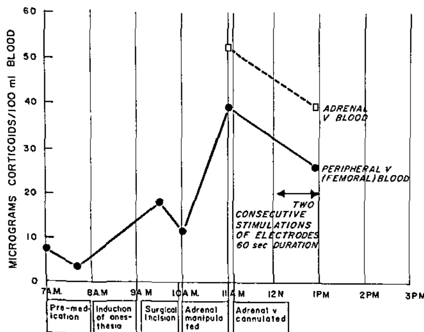


Figure 6

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HYPOTHALAMIC-HYPOPHYSIAL DYSFUNCTION IN MAN: LABORATORY AND CLINICAL ASSESSMENT

CHARLES L. SPURR *and* SAMUEL H. STRIBLING *

THE PRECEDING papers have discussed the inter-relationships of the hypothalamic-hypophysial area from a basic neurophysiologic view. It is the purpose of this paper to reflect the present status of clinical assessment of pituitary function In the care of such patients, it is essential to estimate the degree of involvement by the lesions as well as to provide suitable replacement therapy to sustain the patient through the stress of definitive therapy and re-establish physiologic activity. We shall, first, discuss current means of evaluating the eutropic hormone activity as reflected in the target organs, second, review the techniques to reflect the responsiveness of the neurohypophysis, and, third, present a study of fluid balance mechanisms in a patient.

I

The development of purified hormones of the pituitary have contributed to the improvement in techniques for the evaluation of hypoplasia or responsiveness of the target organs The work of Peters, *et al.* (1954) has demonstrated that failure of the eutropic activity of the anterior pituitary results in hypoplasia of the respective target organs in the following chronological order (1) gonads, (2) thyroid, and (3) adrenals Other changes, as the control of carbohydrate metabolism, are of importance but precise clinical description is limited by our methods of study

Gonadal activity declines promptly following destructive invasion of the pituitary Clinical studies have frequently commented on the occurrence of amenorrhea and declining libido sexualis, usually with decrease in aggressiveness and little con-

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TABLE II

RESPONSE OF THYROID TO THYROTROPIN
RADIO-IODINE I-131 UPTAKE TWENTY-FOUR HOURS

Normal	15 40%
Hypothyroid (Hypopituitary)	6%
Response TSH*	18%

* Thyrotropin 1 3 units every eight hours, total four units

Response of hypoplastic gland to thyrotropin distinguishes hypopituitary lack of eutropic stabilization from primary thyroid deficiency

a patient with a chromophobe tumor symptomatic for nine years The evidence of hypopthyroidism and the response to thyrotropin confirms the lack of eutropic stabilization Very little increase in thyroid I^{131} uptake would occur in hypothyroidism due to primary thyroid disease. Serum protein bound iodine could be used in the same interpretation Conservative replacement therapy with thyroid extract will be discussed later

Carbohydrate Metabolism Dysfunction of the hypothalamic hypophysial integrative response to epinephrine in the control of carbohydrate metabolism and circulating eosinophils may occur promptly in lesions of this area. Hume (1952) has illustrated this by use of the eosinopenic response in animals Unfortunately, the eosinopenic response to epinephrine in the human is not consistent Clinically, we recognize hypoglycemia unresponsiveness as one of the symptoms of lesions in this area. This can be illustrated in Figure 1, in which the insulin sensitivity in the hypopituitary patient is compared to normal, the slow response to hypoglycemia is well illustrated when compared to the normal curve This slow response may be reflected by the use of the technique of Amatuzio (1954), by which the rate of glucose utilization can be compared in the fasting state. This is expressed as removal of glucose in mgm % per minute Figure 1 shows the nomal rate of glucose utilization, 2-4 mgm % per minute, and the normal glucose insulin response, 6-12 mgm % per minute The hypopituitary patient usually shows a normal glucose utilization slope, but less than normal increase in rate when glucose and insulin are given together. The exact mechanism of this characteristic response in hypopituitarism is not clear, but could be

TABLE I

TESTS FOR EVALUATION OF ANTERIOR PITUITARY INTEGRITY

Gonads —	Assay of Gonadotropins
	Endometrial Biopsy
	Sperm Count and Testis Biopsy
Thyroid —	Radio-Iodine I-131 Uptake
	Serum Protein Bound Iodine
	Response to Thyrotropin
Adrenal —	[illegible]

cern on the part of the patient. Assays of gonadotropins are variable and may show low, normal, or increased FSH. It is seldom that an accurate evaluation of the gonads is of essential importance in the care of this group of patients. The method of Gorbman (1945) is most frequently used in the assay of gonadotropins 17-ketosteroids may be of use in estimating the gonadal activity of males as thirty-five per cent or more of urinary 17-ketosteroids are derived from the testis Commonly, clinical evaluation is more representative than laboratory study since it will also aid in chronological evaluation of the lesion. Table I outlines the methods which may be used in eutropic thyroid evaluation. Further discussion of the techniques for gonadal study supply details not essential to this discussion

Thyroid A considerable volume of data is available on the decline of thyroid function in the hypopituitary patient This has demonstrated the unreliability of the basal metabolic rate and cholesterol levels in this type of problem Thyroid status is now rather precisely measured by radioiodine (I^{131}) uptake studies or by the analysis of serum protein bound iodine While it is exceptional for the hypopituitary patient, even marked hypothyroidism, to show classical myxedema, it is of importance to establish the responsiveness of the gland as evidence of secondary *vs.* primary deficiency of thyroid Indeed, recent reports reflect the possibility of failure of individual hormones of the pituitary as thyrotropin (Sampson, *et al*, 1954)

In Table II, we have illustrated the characteristic response of

TABLE III

ASSAY OF ADRENAL CORTICAL FUNCTION
17-HYDROXYCORTICOSTEROID EXCRETION

	Normal 5-12 mgm/24 hr	*Hypoplasia* 2-5 mgm/24 hr.
ACTH (10 mgm I V.)	12–26	6–12
Second Day ACTH	12–26	10–18

given by slow I V. infusion or 40 mgm. ACTH gel. Table III illustrates the normal values and the response of the hypoplastic gland.

Water Metabolism One of the physiologic disturbances of interest for many years has been the disturbed handling of water loads in individuals with adrenal and pituitary dysfunction. The characteristic delay in the excretion of water has been organized by Robinson-Kepler-Power (1941) as a test of adrenal disease In this procedure, the volume of an overnight (10 p.m. to 7 a m.) urine collection is compared to hourly morning specimens after a water load of 20 ml/Kg. of body weight is administered between 7·30 and 8.00 a.m. If the volume of the hourly specimens is larger than the night specimen, adrenal disease is unlikely Frequently, this test is abnormal in pituitary lesions and, after discussing the relationships of the posterior pituitary to water balance, we will consider the complexities presented in attempting to explain the responses seen in the hypopituitary patient

II

In earlier papers of the symposium, we have had several expositions on the neurosecretory mechanisms and their relations to the posterior pituitary. The classical studies of Fisher, Ingram and Ranson (1938) established the structural relationships of the hypothalamus to the function of the posterior pituitary Studies of the neurosecretory phenomenon in man have been few and possibly confused by post-mortem changes. Weicksel and Cain (1952) and Russell and Drager (1954) have been unable to demonstrate neurosecretory material in studies of lesions which have produced diabetes insipidus In normal animals, the neurosecretory material has been clearly demonstrated in the

related to the decrease in the anterior pituitary hexokinase inhibitor resulting in less change in glucose metabolism when insulin antagonizes this inhibitor. Further study will be required to elucidate the details of this mechanism. However, in our studies, this test has been the most reliable means of expressing glucose metabolism. This decreased response to insulin has been seen in twelve patients with various types of pituitary tumor.

Adrenal Cortex: The adrenal status is of paramount clinical and physiological importance in the management of lesions in the hypothalamic-hypophysial area. The response of the adrenal cortex to ACTH has become a standard procedure to establish the functional level of the cortex. Jenkins, *et al.* (1955) have recently evaluated these techniques. The eosinopenic effect of the cortical steroids, manifest four hours following intramuscular injection of ACTH, has been standardized to a degree that it is the simplest assay. Failure of twenty-five units of ACTH to cause a fifty per cent fall in the level of eosinophils requires further study to establish the degree of adrenal cortical dysfunction present. It is now practical to quantitate the level of 17-OH corticosteroids in urine. These include primarily corticosterone and hydrocortone (Reddy, *et al.* 1952). The urinary excretion of this group of steroids will establish the functional level and response to prolonged stimulation as following 10 mgm ACTH

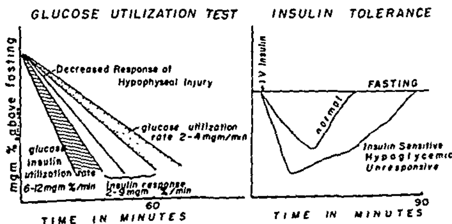


Figure 1 Typical results of glucose utilization test (*Amatuzio*) and the insulin tolerance test in the normal and in hypopituitarism

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hypothalamic hypophysial area by Hild and Zettler (1952) and others.

The occurrence of polydipsia and polyuria with urine of low specific gravity requires evaluation to establish whether we are dealing with psychogenic polydipsia, diabetes insipidus, diabetes mellitus, or chronic nephritis The attempts to withhold fluid are not always helpful to the physician and may accelerate vasomotor collapse. The best technique now available is the study of the *intrinsic antidiuretic response in the hypertonic saline test.* The patient is hydrated with 20 ml. of water per Kg. body weight over a one-hour period. Urine is collected via catheter at fifteen-minute intervals and, when the urine flow exceeds 5 ml per minute, an infusion of three per cent sodium chloride (10 ml/Kg.) is given over a forty-five-minute period. The normal subject will respond to this increase in extracellular tonicity by a sharp reduction in urine flow within the first two fifteen-minute collection periods following the hypertonic saline. In the patient with diabetes insipidus, there is no decrease in volume and, forty-five minutes after the hypertonic saline, 0.1 unit of Pitressin® is administered The decrease in urine volume following Pitressin differentiates diabetes insipidus from chronic nephritis Other groups of investigators have designed tests to meet the specific problems of water load handling.

Further evaluation of polyuria may be obtained by the use of the Nicotine test. The patient is requested to maintain diuresis by drinking water equivalent to the urine flow in fifteen-minute catheter collections. When a flow of 5 cc per minute or larger is obtained, the patient is given, over a five to ten minute period, a dose of 1-3 mgm of nicotine—the higher dose is used in smokers If the patient is a smoker, rapid inhalatory smoking of one to three cigarettes will suffice The normal response to either of these procedures is an immediate antidiuresis with a gradual return of the urine flow within the third or fourth fifteen-minute collection period The patient with diabetes insipidus will show no fall in urine flow. The value of these three tests lies in the aid they offer in differentiating the locus of disturbance in the hypothalamic area, osmoreceptors, or renal tubules. It has also become apparent that some patients with diabetes insipidus have

residual secretory tissue which is responsive to nicotine, but not to the osmoreceptor stimulus of hypertonic saline. Figure 4 demonstrates the response in a patient with a large chromophobe tumor. It will be noted that he does not respond to hypertonic saline, but does respond to nicotine (three cigarettes). Thus, we conclude that neurohypophysial function can be excited by the drug but not through osmoreceptors.

This survey of the diagnostic techniques for the evaluation of a hypothalamic-hypophysial lesion allows us to estimate the degree of dysfunction which has resulted from decreased eutropic stabilization. The development of more potent hormones for replacement therapy has brought not only the benefits of effective treatment but the complications of over-treatment. A progressive increase in reports reflecting this warn that conservative clinical observation paralleling each attempt in substitution therapy is necessary in the care of the hypopituitary patient either in the period of definitive therapy or as substitution therapy is initiated.

The adjustment of thyroid replacement is straightforward, but requires considerable caution as very small doses of thyroid may produce a considerable increment in the tissue metabolism Such increased metabolism and resultant stress has led to sudden death of a significant number of hypopituitary patients through cardiac and adrenal crises (Paull and Phillips, 1954) It is therefore of importance to consider first the replacement therapy necessary for normal adrenal function to meet this metabolic stress This is commonly accomplished by the use of cortisone or hydrocortisone, the former offering a greater degree of sodium retaining effect. Attention should be directed to the fact that here we are replacing physiologic functions and are not striving toward pharmacologic effects as in their use as anti-inflammatory agents. Problems in water metabolism of the hypopituitary patient may be considerably multiplied by the use of large and prolonged administration of steroids. With adequate adrenal replacement therapy underway, the use of thyroid therapy is less hazardous and may, at election, be followed by androgen or other therapy.

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and supportive use of corticosteroids suggest that increased care will be required in the use of adrenal cortical replacement therapy. This problem may be illustrated by the report of a study on a patient who showed this phenomenon

Since 1918, when Von Hamm suggested that diabetes insipidus was possible only if the anterior pituitary was functional, other workers have noted the apparent relationship between the two glands. Recently, a number of cases of co-existing anterior and posterior pituitary hypofunction have been reported by Dingman (1954), Engstrom, *et al.* (1952), and Leaf, *et al.* (1952). The present study is of a patient with a history resembling that of true diabetes insipidus and definite but incomplete anterior pituitary hypofunction. Cortisone consistently produced polydipsia and polyuria, the former being the prime but probably not the initial factor

Methods: Balance studies were done on the metabolic ward of the Veterans Administration Hospital in Houston, Texas Urine was collected every six hours for volume and specific gravity and every 24 hours for other determinations. All blood specimens were drawn in the fasting basal state except for those associated with renal function studies

Sodium and potassium concentrations of serum and urine were determined with an internal standard flame photometer. Urine total solute concentrations were done by the freezing point technique using the Beckman thermometer Serum and urine chlorides were done by the method of Van Slyke and Heiller.* Serum and urine non-protein nitrogen were done by micro-Kjehldahl and urinary ammonia by the method of Folin and Bell.* Urine and blood creatinine was measured by the method of Bonsner, Hereglia, *et al.* (1945), inulin by the method of Roe, Epstein and Goldstein (1949), PAH by the method of Smith, *et al.* (1945).

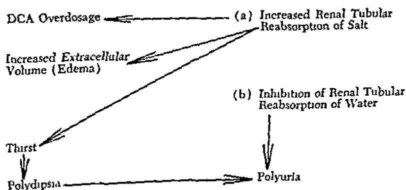
Twenty-four-hour urine collections were concentrated by the method of Grollman (1949) and ADH assays done in duplicate by a modification of the method of Stein, Links and Mirsky (1952) Six groups of four rats were used, each rat weighing from 300 to 400 gm An attempt was made to bracket unknowns be-

* As cited in Consolazio, C. F, R E. Johnson, and E Marek *Metabolic Methods* St Louis, Mosby, 1951, p 140

III

A few minutes ago, we spoke of the inability of some hypopituitary patients to handle loads of water. This is also characteristic adrenal insufficiency and has been studied by Rommelt, Sartorius and Pitts (1949), who thought this to be the result of the inability of the renal tubules to perform the required osmotic work to dilute or concentrate the urine. Gaunt, *et al.* (1949) proposed that there is increased activity of the antidiuretic hormone. This may be due to: (1) over-production resulting from faulty osmoreceptors, (2) delayed destruction, and (3) increased tubular responsiveness. Gaunt favors the view that there may be defective inactivation of the antidiuretic material in the liver since liver slices from adrenalectomized rats inactivated Pitressin more slowly than does normal liver tissue. This is supported further by an increase in the sensitivity of adrenalectomized rats and dogs to Pitressin. If we accept a reciprocal relation in activity between the cortical steroids and the antidiuretic hormone, we can appreciate the sequence of mechanisms in the following diagram adapted from Gaunt (1949).

In 1942, Moehlig and Jaffe reported a diabetes insipidus-like syndrome following implantation of a 250-mgm crushed desoxycorticosterone pellet in a patient with myasthenia gravis. There have been numerous reports of steroid diabetes insipidus in laboratory animals, in most of these, polydipsia has been thought of as primary. The occurrence of polyuria and, indeed, characteristic diabetes insipidus in hypopituitary patients following surgery



Radioactive iodine uptake varied from 1 6% to 9 1% of the administered dose in 24 hours on many occasions. There was an increase to 25 0% in twenty-four hours uptake following 10 mgm thyrotropic hormone. The patient had dry, thin, wrinkled skin and was sensitive to cold

2. Hypogonadism was suggested by 17-ketosteroid excretion in January, 1954 of 1 03 mgm/24 hours. There was loss of axillary and pubic hair and atrophic testes.

3. Hypo-adrenal response varied. 4-hour ACTH test in April 1953 showed 56% fall in eosinophils Later tests showed less response. 11-oxysteroids in January 1954, 3 00 mgm/24 hours, 17-hydroxysteroids, 3 56 mgm/24 hours, September, 1954.

4. Hypoglycemia irresponsiveness was revealed in an I V. glucose-insulin tolerance test in October, 1954 with a drop from 76 to 46 in 150 minutes without reaction

Results: The characteristic handling of water, sodium, and potassium by this patient when influenced by cortisone is shown in Figure 2. The data include intake, output, and total urinary sodium and potassium excretion During this period, the patient was allowed free access to distilled water and 8 gm. of sodium chloride plus 2.4 gm. of potassium chloride were added to the diet. There was an increase in the urine volume which could not be attributed to changes in total or relative excretion of sodium and potassium While the results do not show ideal consistency, there is a transient sodium retention and an increase in potassium output shortly after initiating the cortisone treatment A greater tendency to sodium diuresis follows cessation of the steroid despite a declining urine output. The urine volume increased during each study from an average of 2900 cc. to 5900 cc. during the first trial and to 7560 cc. in the second cortisone study. It then becomes of interest to determine the ability of the patient to handle solutes or the concentrating ability of the kidney.

To evaluate the ability of the renal tubules to concentrate urine, the patient was placed on a liquid formula containing 13 94 gm nitrogen, 1789 8 mgm. (77 8 mEq) sodium, and 2891 mgm (76 9 mEq) potassium and maintained on this for fourteen days It had been found by trial that this amount of sodium was neces-

tween known Pitressin standards, but, due to technical difficulties, no quantitative estimation will be presented.

Case Report: O.M., a forty-six-year-old colored male was admitted to the hospital April 1953 Polydipsia and polyuria were present up to about 8 L/day since 1948. In 1947, he was hospitalized for a year with a diagnosis of paranoid schizophrenia. In 1949, he was given x-ray treatment for a suspected pituitary tumor, with general improvement but no change in the polyuria and polydipsia The patient was able to work as a railroad brakeman until three months before the present admission.

On admission, the patient was lethargic with wrinkled skin, sparse body hair, atrophic testes, and neurological signs indicative of an expanding intracranial lesion. On the third hospital day, a large chromophobe adenoma was partially removed from the region of the pituitary, no attempt being made to enucleate tumor seen posteriorly and superiorly in the region of the hypothalamus A partial frontal lobectomy was necessary for hemostasis.

Four days after operation, with the patient on ACTH (80 mgm/day), a marked increase in urine output was noted, which was controlled with some difficulty with Pitressin At that time, the patient received a second course of 3000 R to the pituitary area. After some fluctuation in serum electrolyte levels, the patient was stabilized on ACTH and Pitressin In October 1953, it was possible to withdraw both if the salt was maintained

Laboratory findings after the patient had been several months without ACTH and Pitressin showed persistently dilute urines, using the specific gravity technique, with a twenty-four-hour volume greater than 3000 cc Serum sodium, chloride, potassium, calcium, phosphorus, blood urea nitrogen and fasting blood sugar were normal. There was a persistent mild normochromic anemia

Serial EEG's made at intervals of several months revealed a disturbance in the normal sleep activity in the left frontal area in addition to left frontal focal signs secondary to surgery

In line with another investigation, the patient was given 100 mgm Cortisone a day, with marked polyuria and polydipsia resulting In view of this, further evaluation of anterior pituitary revealed the following

1. Hypothyroidism was present as demonstrated by basal metabolism rates, −34, −34, −44?, cholesterol, 341, 264, 278, 326

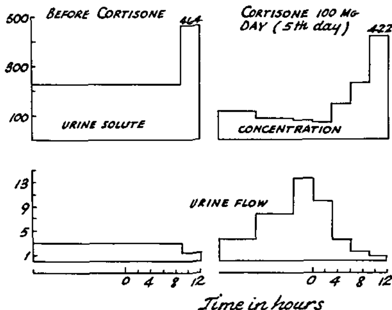


Figure 3 A study of renal tubule concentrating ability of the patient on a constant solute dietary load with restriction of fluid from 9 p m. to 9 a m and solute comparison of 9 p m -6 a m with 6 a.m.-9 a m urine solute concentration Concentrating ability is demonstrated in control and cortisone treatment

hence, reflect neurohypophysial function during the test. This was also shown by the urine flow which decreased from 2 8 to 1.05 cc /minute during the dehydration period before cortisone and from an average of 6.5 cc /minute (maximum was 13 5 cc / min.) to 0 97 cc /min during the cortisone period cited above

Further confirmation of this residual neurohypophysial function is shown by a modified Hickey-Hare test conducted before and after cortisone administration. Figure 4 illustrates the failure of nicotine (three cigarettes, deep and rapid inhalatory smoking) to influence the urine flow but the sharp antidiuretic effect of three per cent sodium chloride and Pitressin, 0.57 milliunits/Kg, I V These data also suggest that neurohypophysial function is responsive and the polydipsia is primary to polyuria

Balance Studies· In an effort to clarify further the cause of this patient's reaction to steroids, studies were done with the pa-

sary to maintain the patient. Four days after this, the patient had equilibrated sufficiently as reflected by the freezing point depression. Food and water were then restricted from nine p m to nine a.m. Total solutes were determined from six p m. to six a.m. and from six a.m. to nine p m. after dehydration. A period on cortisone, 25 mgm. every six hours, was instituted during this period and the procedure was repeated daily. In Figure 3, the data is presented showing a definite increase in solute concentration to 464 milliosmols/L in the pretreatment period and an increase to 422 milliosmols/L when the patient was receiving cortisone These values reflect less concentration than normal maximal levels but indicate a distinct ability to concentrate urine and,

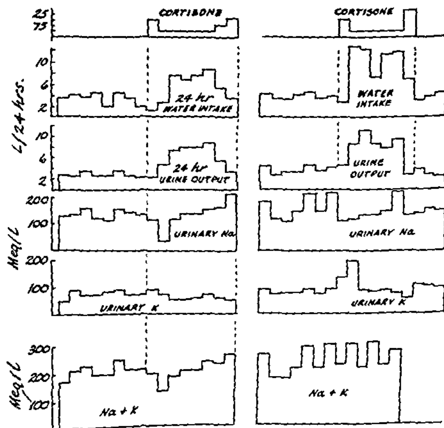


Figure 2 Electrolyte and water balance in hypopituitary patient and effect of cortisone in doses of 25 to 100 mgm daily

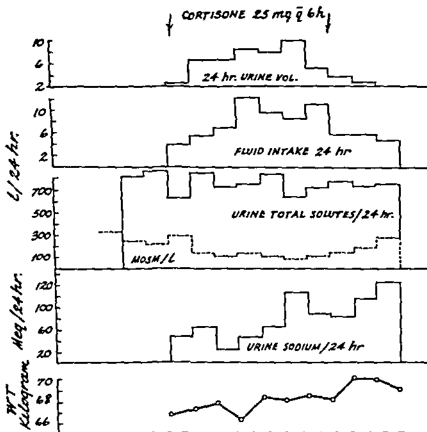


Figure 5 A study of the relationship of fluid intake, urine volume, and total urine solute load when cortisone was given suggests a primary polydipsia

explanation is not readily offered is the increased plasma sodium on the first day of cortisone administration This was confirmed by repeat analyses and also seen on two other occasions with levels of 160 mEq/L on the first day of corticosteroid administration. It suggests the need for consideration of the total compartmentation of sodium as a factor in the mechanism of thirst in this problem

In following the nature of this increased urine flow further, several studies of the discrete renal function were conducted. These are tabulated (Table V) These were done after four days

tient equilibrated on the constant diet and cortisone, 25 mgm. every six hours, was again given (Figure 5). There was no correlation between total solute output and urine volume, the urine becoming markedly hypotonic after Cortisone was administered, but total solute load changing little. This would be expected with a diuresis due to increased drinking water. A depression of the total sodium output occurred early but was apparently compensated for by a rise in nitrogenous material and potassium. The weight gain occurring on this trial may be nutritional, attributed to the constant liquid diet the patient was receiving. The fact that he did not decrease his voluntary fluid intake to compensate for the liquid diet may have some bearing on the reactivity of the hypothalamic thirst mechanism.

The tabulation (Table IV) of endogenous creatinine clearance and plasma electrolytes shows a slight increase in clearance during the period of cortisone therapy. This is not correlated with the urine volume. One of the striking observations for which an

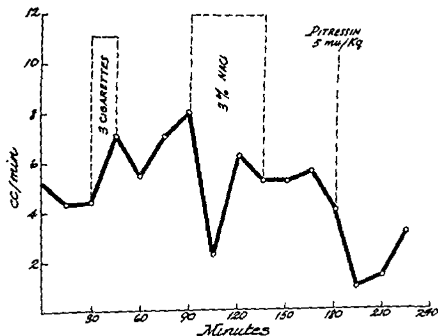


Figure 4 Modified Hickey-Hare test Response of the patient to nicotine, hypertonic saline and pitressin reflects neurohypophyseal response

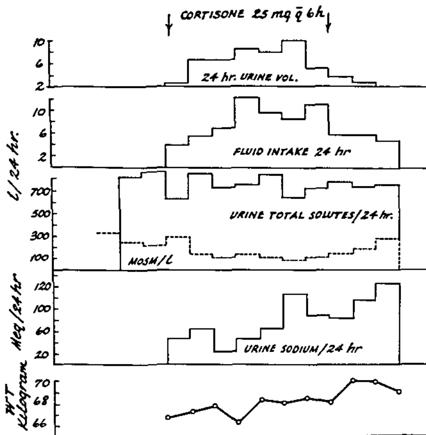


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TABLE IV

TABULATION OF ENDOGENOUS CREATININE CLEARANCE, PLASMA ELECTROLYTE LEVELS, NON-PROTEIN NITROGEN AND HEMATOCRIT. *Cortisone, 25 mgm every six hours, was given from the twentieth through the twenty-second.*

Date	*Blood Creatinine*	*Urine Creatinine*	*Clearance ml/min*	*Blood Na*	*Blood Cl*	*Blood K*	*NPN*	*HCT*
18		1 01						
19	1 16	1 10	65	130	96 6	4.3	31 6	34
20	.86	1 18	95	153	92 6	4 6	51 5	34 5
21	1 20	1 16	60	137	104 6	4 3	63 6	33
22	86		94	136	99 3	4 2	50 3	31
23	1 13	98	77		108 4			
24		1 11						
25		1 01						
26	1 22	.90	58	144	96 6	4 5		31 5
27	1 12	.96	56	141	95 6	3 9	36 1	32 5
28	90				95 2		48 7	33

TABLE V

TABULATION OF DISCRETE RENAL FUNCTION TESTS PRIOR TO STEROID THERAPY AND FOLLOWING CORTISONE AND INTRAVENOUS HYDROCORTISONE. THE DEPRESSED FUNCTION OF HYPOPITUITARISM AND THE PARTIAL IMPROVEMENT WITH CORTICOSTEROIDS WITH RESPONSE TO PITRESSIN IS DEMONSTRATED

		$GFR_{(in)}$	$GFR_{(CR)}$	*RPF*	*FF*	*RBF*	*TmPAH*	*cc min*
1/24		71	69	506	14	766	86	2 8
4/23	E 25 mgm po x4 days	79	79	940	09	1347	78	7 2
	2 mu pit IV	73	58				92	2 8
6/9	F 100 IV	92	84	658	14	1020		10 4
	2 hrs before IV pit	60	60	471	13	731		1 3
6/17	2% alcohol IV	57	56	396	16	577		1 4
	2 hrs before IV pit	47	37	368	13	533		1 0

treatment with cortisone, 25 mgm. each morning. The moderate increase in clearance effected by cortisone is seen, but normal levels are not attained. This is characteristic of the patient with anterior pituitary insufficiency. Pitressin was given two hours *later to demonstrate the renal responsiveness* *Later a study was* done with intravenous hydrocortisone, 100 mgm. given over a two-hour period, completed one hour before the renal function test. The second test with Pitressin confirmed the hemodynamic effects of this agent in the presence of hydrocortisone. A third set of tests shows the effect of two per cent alcohol alone since this solvent was used in the hydrocortisone test. Through all of these studies, an oral intake of 200 cc. of water was begun four hours before the test and maintained to insure a moderate water load. The more marked effect on the clearances by hydrocortisone was accompanied by a rapid increase in urine flow The response to Pitressin was not altered by the corticosteroid. Alteration in renal function does not explain the increased urine volume produced by steroids.

Assays for antidiuretic hormone (ADH) were made on two twenty-four specimens from each period of a controlled solute level study. The concentrates during the control period showed twice the activity of those collected during the period of steroid therapy.

Discussion. Detailed attention was given to the study of this patient because of the repeated clinical observation that, with the use of ACTH or cortisone, we were seeing periods of diuresis and return of diabetes-like syndromes in hypopituitary patients following definitive therapy. It appeared that these were related primarily to the steroid However, it is likely that the mechanism may vary in each patient. In the one discussed here, it appears that a stimulus to thirst may be the primary change. This is in keeping with the concept of Gaunt (1949), referred to in introducing this study. Hence, the increase in serum tonicity with sodium retention results in thirst and polydipsia leads to polyuria The ADH assay suggests that the decreased level seen when cortisone was given may be sufficient to play a part in the water diuresis.

SUMMARY

A survey of methods of study of the anterior and posterior pituitary have been presented. Clinical implications of these studies are further reflected by the presentation of a case of hypopituitarism in which a diabetes insipidus-like syndrome was produced by the corticosteroid replacement therapy. The importance of the corticosteroids in the water balance of hypopituitary patients is emphasized.

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GENERAL DISCUSSION AND SUMMARY

Hoff: There is one problem on which I would like for our experts to come up with a little more clear-cut answer. It seems to me that I have seen two currents of thought developed this afternoon and this morning regarding the control of the anterior pituitary. One seems to indicate utilization by the anterior pituitary of mechanisms which apparently focus in the posterior pituitary; that is, the secreting neurons that Dr. Hild mentioned which, from the evidence of Dr. Stein, seem to participate, not only in the regulation of secretion of the posterior pituitary, but also the anterior. On the other hand, from the evidence brought forward by Dr. Harris this would not seem to be the case. I wonder if you would start the discussion, Dr. Guillemin, by discussing what you think of the relationship of the posterior pituitary neural secretory mechanisms to any hypothetical anterior pituitary neuron secretory mechanism.

Guillemin It is certainly extremely interesting to find such a high degree of correlation between ADH activity and ACTH release. The results reported by Dr. Stein are indeed most evocative of some relationship between release of ADH and activation of the anterior lobe of the pituitary upon exposure to stress. However, it seems to me that we are still missing the demonstration of a causative relationship between these two events The release of ACTH produced by injection of commercial extracts of the posterior lobe of the pituitary (Pitressin) should not be taken as a final proof of this point for two reasons. ACTH release is the most "non-specific" response which, by definition, is elicited by any "non-specific" agent Secondly, commercial Pitressin contains, in addition to vasopressin, a number of other constituents as shown by electrophoresis (Taylor, S P., duVigneaud, V., and Kunkel, H. G.: *J Biochem*, *205* 45, 1953) or by chromatography (Housholder and Guillemin, *unpublished*). It is therefore difficult to attribute Pitressin-induced ACTH release specifically

to vasopressin. The most direct approach to this problem is the type of *in vitro* studies we have reported, where the pituitary can be directly stimulated and its ACTH release directly estimated. We thus could confirm the ACTH releasing activity (McCann, S M., and Brobeck, J. R . *Proc Soc Exper. Biol. & Med*, 87· 318, 1954) of commercial Pitressin even at a very low dose level (one unit per pituitary per 48 hours of culture, i e., less than twice the total vasopressor activity of one posterior lobe of a rat pituitary). This ACTH hypophysiotrophic activity in our *in vitro* system was not shared by purified vasopressin, kindly supplied by Dr. V. duVigneaud These results we would interpret as the proof that "something" else other than vasopressin, in Pitressin, some contaminant likely of hypothalamic origin, stimulates directly the pituitary to release ACTH.

It might be interesting to report here the results of several experiments we performed recently in which we studied "adaptation" to Pitressin. As shown long ago by Selye, upon continuous exposure to a stressing agent, there soon occurs a state in which there is no more sign of ACTH release when the stimulus is applied. The mechanisms underlying this "adaptation" are not known It is neither a pituitary nor an adrenal phenomenon since upon exposure to a different kind of stress, ACTH is immediately released, though in a somewhat lesser amount (Guillemin, unpublished) An alternative explanation would be that this "adaptation" takes place at the level of the nervous link, that is the hypothalamus, in the neuro-endocrine system stimulated by stress It might involve inhibition of the neural mechanism which releases the hypothalamic hypophysiotropic mediators. Now, if one was to inject chronically the hypothalamic substances responsible for ACTH release, one would then expect that continuous secretion of ACTH could be demonstrated We have injected subcutaneously, twice daily for fourteen days, 2 5 U of Pitressin to rats of 100-150 gm. body weight The drug was dissolved 1/1 in a slow absorption medium of polyvinylpyrolidones (Subtosan, Poulenc Ltd., Montreal, Canada) The results show that there is no adrenocorticotrophic adaptation to Pitressin In other words, the animals release just as much ACTH (as judged by the adrenal ascorbic acid depletion test) after fourteen days of administration

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the hypothalamus of the hypophysectomized mother rats. The transplanted material became vascularized by the portal vessels and normal anterior pituitary function was re-established However, we noticed that although the hypophysectomized female rat bearing a pituitary transplant would mate, become pregnant and deliver living young, that such young died unless the mother received repeated injections of oxytocic hormone. The oxytocic hormone is of course necessary to cause ejection of milk already present in the mammary glands to the suckling young. The point I am trying to make here is that there was again an absence of oxytocic secretion, indicating a lack of posterior pituitary function in the presence of an apparently normal activity by anterior pituitary tissue. Then, as I have mentioned before, there is a difference in the hypothalamic sites at which electrical stimulation will evoke discharge of antidiuretic hormone or ACTH In our experiments, and in those of some other workers, stimulation of the more posterior part of the hypothalamus is most effective in causing discharge of ACTH, whereas stimulation of the anterior supraoptic region is most effective in causing discharge of the antidiuretic hormone. I think the data from these experiments, conducted *in vivo,* as well as from the *in vitro* experiments from Dr Guillemin, the evidence is against the view that the antidiuretic hormone is the humoral transmitter involved in ACTH discharge

Stein This is not a new problem We discussed it yesterday in some detail, certainly most of our evidence is by inference and I think the critical experiment has not yet been designed or done. We attempted to do this we attempted to adapt rats to small graded doses of ADH to study the effect on the adrenal with the hope that we would not get an antidiuretic effect In other words, we were trying to adapt them to the antidiuretic effect Perhaps antidiuresis in itself may cause adrenal hypertrophy We were not successful

Guillemin What happened to the weight of the adrenals?

Stein We got a slight increase in the weight of the adrenal, but on the other hand, we had a persistent antidiuretic response. There is one thing I am confused about in your study *in vitro.* It seems to me that there is a marked variability in the ACTH content of the fluids of your control pituitary glands at the second

of Pitressin as they do after the first injection. This effect cannot be accounted for by the ACTH contamination present in the dose of Pitressin utilized here. These experiments we feel have more significance for a special action of Pitressin in ACTH release than the observation of the corticotrophin release which follows one single injection of Pitressin. They seem to point out that Pitressin behaves differently than other drugs regarding ACTH release. It would be interesting to see, especially in view of our *in vitro* results, if purified vasopressin would give different results, i.e., produce "adaptation." * The ACTH hypophysiotrophic activity of commercial Pitressin could be explained if one of the contaminants of vasopressin in it were the still hypothetical hypothalamic mediator of ACTH release.

In summary, I would like to say that the relationships between activity of the posterior lobe of the pituitary and release of ACTH by the anterior lobe, as observed by Dr. Stein and Dr. Mirsky, are remarkable and undoubted. However, it does not seem to me that we can implicate vasopressin as the sole and exclusive mediator for ACTH release from these experiments and observations. The results of our *in vitro* studies are indeed in contradistinction with this hypothesis.

Harris: I would like to add one word in agreement with Dr. Guillemin. There is evidence that destruction of the supraoptico-hypophysial tracts in the hypothalamus, resulting in marked diabetes insipidus, does not necessarily disturb the functions of the anterior pituitary gland. In such experiments there is disappearance of the secretion of the antidiuretic hormone and an intact secretion of anterior pituitary hormone, which would indicate that the antidiuretic hormone does not play a major role in the function of the anterior pituitary. A similar conclusion was drawn from the results of experiments performed in collaboration with Dr. Jacobsohn (1952) ** In this work we transplanted the pituitary glands of newborn rats to a site beneath

* The experiment which was completed after this symposium showed that indeed there is "adaptation" to the non-specific adrenocorticotrophic effects of purified vasopressin (Guillemin and Housholder, to be published).

** Harris, G. W., and Dora Jacobsohn. Functional grafts of the anterior pituitary gland. *Proc. Roy. Soc., London, sB*, *139* 263-276, 1952.

intake or urine output of our pituitary-transplanted rats, so we do not know whether they had a diabetes insipidus. I agree with Dr. Green in his line of argument that it is only on the assumption that the oxytocic and antidiuretic hormones are secreted simultaneously that one can bring forward our pituitary-transplanted rats as evidence against the view that the antidiuretic hormone is involved in ACTH secretion. However, there is the recently published work of Drs. McCann and Brobeck (1954),* in which they found rats that had maximum diabetes insipidus also showed adrenal hypertrophy.

Dr. L. Kraintz, Houston, Texas I do not want to detract from Dr. Guillemin's brilliant work, but I think there is something we cannot overlook and that is the purified vasopressin in your experiments I think we cannot assume that it represents the circulating hormone. The nature of this circulating hormone may be proteinic as Van Dyke has shown and you may be just testing one moiety of this protein, that is the vasopressin polypeptide. I think this is a false assumption that what we assay in the test tube in a pure form is the circulating hormone.

Green: Dr Hoff has asked me to discuss Dr. Gloor's paper. I think the most remarkable thing about Dr. Gloor's paper is that here is another example of the telepathic process that goes on between Los Angeles and Montreal There seems to be a continual flight of ideas between both places and these two flights of ideas often coincide very closely One thing we have been investigating a little lately has been the interference between two shocks applied to the hippocampus. We find that we can potentiate hippocampal responses by repetitive stimulation and that the potentiation then persists for quite a long period I wonder if, in Dr. Gloor's view, this represents a recruitment of neurons It seems to be analagous to the situation he ascribed to the amygdala, and I think probably one would find that the two types of effect were just about the same Do you ascribe this to some kind of recruitment, or perhaps to some ionic change that is going on in the tissues immediately adjacent to stimulation, and how far do

* McCann, S M, and J R Brobeck Evidence for a role of the supraoptic hypophysial system in regulation of adrenocorticotrophin secretion. *Proc. Soc Exper Biol*, 87 318-324, 1954

day of the cultures. How do you account for these wide discriminations here?

Guillemin: I have no good explanation for the discrepancies in the basal ACTH activity found in the culture fluids from one experiment to the other. At the neutral pH by which we must abide in these organs or tissue cultures, there is a non-negligible inactivation of ACTH; here might be at least a partial explanation for these variations It might also be that these discrepancies are inherent to the glands themselves from one experiment to the other. Pincus has noted similar variations in the basal levels of corticoid secreted by their adrenals *in vitro*. I am not too unhappy about these variations as long as we know they exist and as long as we constantly have for each experiment one control group of pituitaries Whenever we note an increase in ACTH release, with for instance hypothalamic extracts or Pitressin, this is always referred to the basal level secretion of the control group

I was very interested in your experiments on a possible adaptation to the effects of antidiuretic hormone In our study where Pitressin was injected up to fourteen days and where ACTH was constantly released as judged by adrenal ascorbic acid depletion, there was no hypertrophy of the adrenals, much to our surprise.

Green I wonder if I can raise an anatomical point, probably in the form of a question to Dr Harris I wonder whether Dr Harris' rats, which he mentioned a moment ago, had diabetes insipidus This seems to me to be a point in this connection, since hypophysectomy does not normally produce diabetes insipidus

The second thing is this, that the median eminence, since it has a completely independent blood supply from the neural lobe, could conceivably be acting in some entirely different way from the neural lobe itself Could this tie in perhaps with the difficulty in milk let-down that you mention? Is a larger amount of posterior lobe hormone necessary for this particular function?

The other difficulty raised—the difficulty of stimulating the supraoptical hypophysial tract and failing to see anterior pituitary effects, and the difficulty of Ranson's observations I cannot account for, but I would just like to ask about this one point here

Harris Dr Jacobsohn and myself did not measure the water

On the other hand, I think that repetitive stimulation is probably closer to what happens normally than single shock stimulation. After all, in the normal organism excitation will be repetitive though of course not in the regular, monorhythmic way as in our artificial experimental situation.

Hoff: I wonder if Dr. Gloor or Dr. Green, or both, would help us with Dr. Pool's problem of finding that stimulation might diminish secretion. This is a question I put to Dr. Magoun in another connection, asking him whether he thought that in the upper reaches of the brain stem there were inhibitory or suppressor circuits akin to those which descend and which match an activating circuit. Dr. Gloor talked about activating circuits which might affect the pituitary. Do you think this might be a matter of latching onto a suppressor circuit, or just that you have probably disrupted the activating circuit by your stimulus?

Gloor: I do not know, but I think it is another example of the puzzling results you get from systems which somewhat duplicates what we have with respiration, motor function, blood pressure, etc., where one can obtain activation or inhibition. I do not know exactly if it is the same thing here with these endocrine effects. I think we should have more experience and experiments with animals where we can control the conditions much better than we can with humans

Green I would not care to make any serious comments. This reminds me somewhat of a discussion that went on for many years between Klüver and Bucy on one hand and Bard and Mountcastle on the other with regard to central nervous lesions and the behavioral changes which occurred. I suppose that now one would think that Bard and Mountcastle probably removed a little bit more and damaged the hypothalamus, and that perhaps there is here some interplay between the amygdala and the nuclei of the hypothalamus. Maybe this is analagous, but I would not like to comment further.

SUMMARY OF DAY'S SESSIONS

G W. Harris The problem of trying to summarize the wealth of material that we have had presented today seems to me no easy task It might be possible to summarize in diagrammatic

you think that these responses which both of us obtained in this way can be regarded carried over physiological pathways, in that we have to put in a series of repetitive volleys to obtain them. I think in general we are in very close agreement indeed, not only on our data, but on our speculations about our data.

Gloor: I think we are unable at the present to make any definite statement concerning the mechanism and the site of this potentiation effect. However, I have the feeling that it does not take place in the amygdala itself For if we record from the areas which we know are directly connected to the amygdala itself, as e.g. the lateral preoptic area, or if we record from the stria terminalis, which is one of the main pathways from the amygdala, we do not find any potentiation; or at least it is very minimal. On the other hand, the responses with the longest latencies, e.g., in the hippocampus, give the most profound potentiation effect. Thus I have the idea that it may be something which goes on along a chain of neurons. The process underlying potentiation may eventually start, to a certain extent already, in the amygdala itself because we may have a very minimal degree of potentiation in directly related structures, but it is somehow amplified by every synaptic passage I cannot prove that I am right, but I think the evidence we have so far would not contradict such a view but rather support it. Another evidence which indicates that some potentiation process is already starting at the level of the amygdala itself is evidenced by the following observation. If we use short pulses to stimulate the amygdala, e g. 0 25 msec pulses, even if we use quite high voltages, we do not get any response in the hypothalamus. If we use long, e g, 5 msec. pulses, we obtain a good response. Now, if we stimulate the amygdala repetitively with long pulses and then, when switching back to single shock pulses, instead of using long single shock pulses, we use short pulses, we now do obtain a potentiated response, which means that at the site of stimulation itself a change must have taken place.

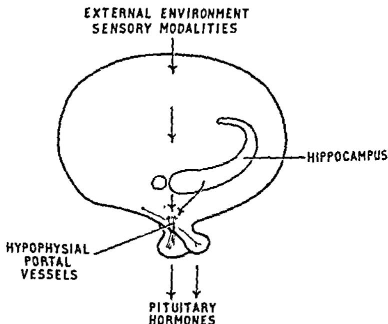
The second question asked by Dr Green, whether the po-

[illegible]

about that too I think one ought to keep this possibility [illegible]

relating the hippocampal and amygdaloid structures to the hypothalamus is opening up a new field of research. I myself would think that the amount of work devoted to the connections both anatomical and physiological between the hippocampus and the endocrine system is going to increase rapidly in the near future.

Passing down this diagram, we had the paper of Dr. Hild and saw his fascinating film dealing with cultures of the hypothalamus and the posterior pituitary gland. There was one question that I wanted to ask Dr Hild, but failed to do so in the very active discussion that followed his paper. My question was this Whether there was any data from tissue culture experiments relating the paraventricular nucleus to the oxytocic hormone and the supraoptic nucleus to the antidiuretic? There is evidence in the form of preliminary communications by Dr. Cross (1955) in Cambridge and Dr Olivecrona (1954) in Sweden that such may be the case It would be interesting to see whether the neurosecretory material from the paraventricular nucleus varied with different physiological states known to be associated with oxytocic secretion, in the same way that it has been shown that the neurosecretory material in the supraoptic system varies with the discharge of antidiuretic hormone. There is one point that I would like to comment on in this respect, and that is that the number of hormones discharged by the posterior pituitary gland are not yet exactly known We have the classical work of the duVigneaud group (1953) in which the chemical formula of the oxytocic and antidiuretic substances have been obtained and in which one of these polypeptides has been synthesized However, it seems to me that the evidence is still incomplete that these substances pass, as such, into the blood stream However, if the paraventricular nucleus regulates the release of the oxytocic hormone and the supraoptic nucleus regulates the release of the antidiuretic hormone, the position would be clarified The point that the antidiuretic hormone may be involved as a humoral transmitter in the hypophysial portal vessels regulating the activity of the pars distalis of the anterior lobe received some discussion here today This point was studied by Dr Guillemin with his beautiful *in vitro* studies, utilizing tissue cultures of the anterior pituitary Possibly in fifty years time endocrinologists interested



form the systems that we have been discussing (draws diagram on blackboard) The systems that we have been studying all day seem to form a hierarchy involving the effect of the external environment on the central nervous system, the pathways by which the various sensory modalities affect the hypothalamus, hippocampus, and amygdala, the anatomical pathways subserving the interplay between these and the hypothalamus, and the pathway from the hypothalamus to the posterior pituitary and the anterior pituitary glands I was interested that both Dr Gloor and Dr Green mentioned the evolutionary aspect to this problem of hypothalamic and pituitary interrelationships, as did our Chairman in his opening remarks, when he recalled the fact that the hypothalamus is related to two great systems regulating the internal environment, that is, the autonomic system and the endocrine system. Clearly the internal environment is a matter of consequence to vertebrate animals and the particular mechanism situated at the base of the brain, the hypothalamo-hypophysial system, is as has been emphasized by Professor LeGros Clark (1938) a constant anatomical feature in vertebrates from the fish upwards. The further data discussed by Dr Gloor and Dr. Green

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in the pituitary gland will be dealing more with test tubes than with intact animals. Regarding the mechanism of anterior pituitary activation, my own feeling at the moment is that the situation can be summarized by stating that the portal vessels of the stalk are clearly and intimately related to anterior lobe activity but that the question of a humoral transmitter carried by these vessels will only be decided when a particular activating substance can be extracted from the blood carried by these vessels, and when the concentration of that substance in different physiological conditions can be shown to vary with the functional activity of the gland. This is a difficult problem since it is a technical achievement to obtain blood from the portal vessels We have been interested in this aspect, but find we only obtain a volume of 0.3 to 0.5 cc of blood from the portal vessels of the cat in half an hour One is handicapped therefore by the small quantities of blood obtainable for analysis.

The interesting suggestion made by Dr. Stein in his paper regarding the relationship between the neurosecretory material described by Dr. Hild and the discharge of ACTH from the anterior lobe of the pituitary certainly deserves further investigation. The evidence from some directions would seem to be against this point of view, but the suggestion should clearly be explored further. The other point that Dr. Stein emphasized was the close relationship between the discharge of posterior pituitary hormones and stressful procedures, the variety of stress stimuli in the experimental animal that will cause discharge of antidiuretic hormone, and the fact that similar stresses in the human—painful stresses—will also cause similar secretion. In this type of work the neurohypophysists are in a better position than the adenohypophysists in the respect that they are making direct assays of the hormone in the blood stream If it were only possible to assay anterior pituitary hormone in the venous blood drained from the anterior pituitary blood of the conscious animal, I believe that our knowledge of endocrinology would change overnight This matter of the method of assay and the method of measuring endocrine activity in the human was emphasized by Dr. Spurr in his talk. The method of Dr. George Sayers (1953) and his co-workers in assaying the blood level of ACTH perhaps offers hope

that methods will be worked out in the near future to deal with the other anterior pituitary hormones.

One point with which I could not quite agree with Dr. Spurr was his statement that the experimentalist was, in this field, so far ahead of the clinician. One could bring a strong argument in the opposite direction; for example, the great stimulus that laboratory investigations received following the discovery of the action of ACTH and cortisone in the treatment of rheumatoid arthritis, and one could go further afield and point to the many accounts in the clinical literature describing the small localized lesions in the region of the posterior hypothalamus which have resulted in precocious puberty (Weinberger and Grant, 1941, Bauer, 1954). These are now well authenticated cases, and yet as far as I know, no experimental worker has yet placed lesions in the posterior hypothalamus of young animals to investigate any change in the time of onset of puberty. It would be possible to continue pointing out examples where the clinical investigator is still offering observations of immediate interest in the laboratory, but perhaps the important point is that both the clinician and the experimentalist are hard at work in this field of pituitary-hypothalamic relationships. There is encouragement and mutual intellectual stimulation coming from both the wards and the laboratories, one adding to and stimulating development in the other.

Regarding the fascinating paper of Dr Pool, I have many questions myself that I would like to put to him I think, however, that this is not the proper moment, but I hope to catch him at a later time and find out a great deal more about the techniques he uses and the results he has obtained

There remains for me now only the very pleasant task, and one in which I am sure all the guests of the Houston Neurological Society will join me, of thanking the Houston Society and Dr Elliott, Dr. Greenwood, Dr Hoff, and Dr Carton and their colleagues in making this day possible for the most interesting discussions that we have had and also for the warmth of their splendid hospitality.

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A Symposium

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